03-12-08 For AF

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:

Rea et al.

Serial No.: 09/666,430

Filed: September 21, 2000

For: DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

Confirmation No.: 6289

Examiner: F. Pierre VanderVegt, Ph.D.

Group Art Unit: 1644

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BRIEF ON APPEAL

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

This brief is submitted as a single copy pursuant to 37 C.F.R. § 41.37 and in the format required by 37 C.F.R. § 41.37(c) (1):

(1) REAL PARTY IN INTEREST

The real party in interest in the present pending appeal is Leids Universitair Medisch Centrum (University of Leiden Medical Center), assignee of the pending application as recorded with the United States Patent and Trademark Office on October, 2000, at Reel 011197, Frame 0136.

(2) RELATED APPEALS AND INTERFERENCES

Neither the Appellants, the Appellants' representative, nor the Assignee is aware of any pending appeal or interference which would directly affect, be directly affected by, or have any bearing on the Board's decision in the present pending appeal.

(3) STATUS OF THE CLAIMS

Claims 1, 40-49, 51-53, 55, 56, 58, 59, 61-63 and 65-68 stand rejected.

Claims 50, 54, 57, 60 and 67 are objected to.

Claims 64 and 69-81 are allowed.

The rejections of claims 1, 40-49, 51-53, 55, 56, 58, 59, 61-63 and 65-68 are being appealed.

(4) STATUS OF AMENDMENTS

The appellants' amendments, filed April 27, 2005 in conjunction with a Request for Continued Examination, have been entered.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention provides means and methods for immunotherapy. The invention provides immune cells and methods to generate them, where the immune cells have the capacity, at least in part, to reduce an immune response in a host. *See*, Substitute Specification, mailed March 18, 2003, at page 6, lines 22-29. In one aspect, the invention provides a method for generating a dendritic cell with the capacity to tolerize a T-cell for the antigen the T-cell is specific for. *Id.*, at page 6 line 22 through page 7, line 12. More specifically, one aspect of the invention relates to culturing blood monocytes from a subject to differentiate into dendritic cells, activating the dendritic cells in the presence of a glucocorticoid hormone, and loading the

activated dendritic cell with an antigen that a T-cell is specific for. *See*, Specification at *Id.*, at page 6 line 22 through page 7, line 24; page 8, lines 1-9; page 11, lines 6-24.

As set forth in 37 C.F.R. 41.73 (c) (1) (vii), every means-plus-function claim must be identified and the structure materials or acts described in the specification corresponding to each claimed function must be set for the with reference to the specification. The current application contains a single means-plus-function claim, to wit: claim 1. The relevant means-plus-function language of claim 1 recites "activating said dendritic cells with a means for reducing IL-12p40 production by said dendritic cells."

The specification, in Example 3 (*See*, Substitute Specification, mailed March 18, 2003, at page 10, line 19 through page 11, line 2.), clearly indicates that dexamethasone, a known compound, has the ability to reduce reducing IL-12p40 production by a dendritic cell, and is thus a disclosed means to accomplish this function. Further, the Patent Office, in the Office Action mailed July 26, 2005, at Page 5, agrees dexamethasone is a disclosed means for the function of reducing IL-12p40 production by said dendritic cells.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether claim 1 is unpatentable under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement?

B. Whether claim 1 is unpatentable under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention?

C. Whether claims 40-49, 51-53, 55, 56, 58, 59, 61-63, 65-68 are unpatentable under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement?

(7) ARGUMENT

A. Claim 1 complies with the written description requirement of 35 U.S.C. § 112, first paragraph.

Alleged basis of rejection

In the Office Action of October 9, 2007, the Examiner rejected claim 1 as assertedly insufficient under the written description requirement of 35 U.S.C. § 112, first paragraph. The Examiner asserted,

> (claim 1) contains subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention... [T]here is insufficient written description to show that Applicant was in possession of 'means for reducing IL-12p40 production by said dendritic cell.' As said 'means' comprise a genus of indeterminate size, one of skill in the art must conclude that the specification fails to disclose an adequate written description or a representative number of species to describe the claimed genus. 10/09/07 Office Action, at page 2, line 28, through page 3, line 5.

Claim 1 complies with the written description requirement, because (I) claim 1 is a "means-plus-function claim" entitled to examination under 35 U.S.C. § 112, ¶ 6, and (II) claim 1 satisfies the two part test established by the United States Patent and Trademark Office to establish compliance with the written description requirement by means-plus-function claims.

> A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, para. 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-function limitation. MPEP 2163 II(A)(3)(a).

Claim 1 is a "means-plus-function claim" entitled to examination under 35 U.S.C. § 112, ¶ 6, in part because the Applicants have used the phrase, "means for," modified that phrase by the functional language, "reducing IL-12p40 production by () dendritic cells," and not modified that phrase by any structure, material, or acts for achieving that function. And, claim 1 complies with

the written description requirement, in part because the Applicants have linked a structure, e.g. dexamethasone, a glucocorticoid, to the function of reducing IL-12p40 production by dendritic cells in a manner that makes it clear to those skilled in the art that dexamethasone is a structure linked to that function.

I. Claim 1 is entitled to the benefit of the examination under 35 U.S.C. § 112, sixth paragraph.

Congress has allowed by statute for patent applicants to claim subject matter according to a means for performing a specified function.

> An element in a claim for a combination may be expressed as a means or step for performing a specified function without the recital of structure, material, or acts in support thereof, and such claim shall be construed to cover the corresponding structure, material, or acts described in the specification and equivalents thereof. 35 U.S.C. § 112, sixth paragraph.

The United States Patent Office and the courts have established a test for according patent applicants the benefit of reciting a claim limitation using the "means-plus-function" language allowed under 35 U.S.C. § 112 ¶ 6. That test, as provided in MPEP 2181(I), is,

> A claim limitation will be presumed to invoke 35 U.S.C. 112, sixth paragraph, if it meets the following 3-prong analysis: (A) the claim limitations must use the phrase "means for" or "step for;" (B) the "means for" or "step for" must be modified by functional language; and (C) the phrase "means for" or "step for" *must not be modified by sufficient structure, material, or acts for achieving the specified function.* Emphasis added for clarity.

Applicants are entitled to the benefit of 35 U.S.C. § 112 ¶ 6, because claim 1 clearly satisfies the 3-prong analysis set forth by the Patent Office. The Examiner has not challenged the fact that the claim recites a "means for" performing a function, or the fact that the "means for" is modified by functional language. Office Action 10/09/07, p. 3. Rather, the Examiner relies on the third prong of the analysis, which is asserted as, "[t]he third prong of 112, 6 states that the claim shall be construed to cover the corresponding structure, material, or acts described in the specification and equivalents thereof." Office Action 10/09/07, p. 3. (internal citation omitted).

The Examiner refused to accord claim 1 the benefit of 35 U.S.C. § 112 ¶ 6, as failing to satisfy this "third prong." As drawn from the MPEP above, the third prong set forth by the Patent Office is "the phrase 'means for' or 'step for' must not be modified by sufficient structure, material, or acts for achieving the specified function."

Independent claim 1 recites "a method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host, said method comprising: culturing peripheral blood monocytes from said host to differentiate into dendritic cells; activating said dendritic cells with a means for reducing IL-12p40 production by said dendritic cells; loading said dendritic cells with an antigen against which said T-cell response is to be reduced; and forming a pharmaceutical composition comprising said loaded, activated dendritic cells for administration to said host." The element in question is "a means for reducing IL-12p40 production by said dendritic cells." The Examiner has not alleged that the phrase "means for" is modified by any structure for achieving the specified function of "reducing IL-12p40 production by said dendritic cells." There is no structure, material, or act recited within the element that could modify the phrase "means for." Production of IL-12p40 is a cellular process. By the express language of claim 1, reducing the operation of this process in a particular cell, the dendritic cell from step 1 of the claim, is the function for which the means is claimed. The specification identifies dexamethasone as a structure corresponding to this means, and dexamethasone is not imported into the language of the claim. Consequently, this element of claim 1 is subject to examination as a "means-plus-function" element, because it meets all three prongs of the Patent Office's analysis.

II. Claim 1 satisfies the written description requirement under the test established for examination of means-plus-function claims.

Once a claim is entitled to examination as a means-plus-function claim, it still must be analyzed "to determine whether there exists corresponding adequate support for such claim under 35 U.S.C. 112, first paragraph. MPEP 2181(IV).

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact

> terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention. 35 U.S.C. § 112, first paragraph.
>
> A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, para. 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-function limitation. MPEP 2163 II(A)(3)(a).

In the Office Action mailed on 10/09/07, the Examiner stated that "[a]s said 'means' comprise an unknown genus of indeterminate size, one of skill in the art must conclude the specification fails to disclose an adequate written description or a representative number of species to describe the claimed genus." Office Action at page 5, lines 2-5. By this, the Examiner implies that the Applicants are attempting to claim a genus, and are required to disclose a representative number of species to describe that genus. This is incorrect, and it is inconsistent with the examination guidelines for claims containing "means-plus-function" limitations under 35 U.S.C. § 112 ¶ 6. Claim 1 is drawn to a method for preparing a pharmaceutical composition that contains a step of "activating said dendritic cells with a means for reducing IL-12p40 production by a dendritic cell." As the element of "a means for reducing IL-12p40 production by a dendritic cell" is a "means-plus-function" element, it satisfies the written description requirement of 35 U.S.C. § 112 ¶ 1 if "[t]he written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation." MPEP 2163 II(A)(3)(a).

Applicants have adequately linked the structure of dexamethasone (DEX) to the function of reducing IL-12p40 production by a dendritic cell. "The proper test for meeting the definiteness requirement is that the corresponding structure of a means-plus-function limitation must be disclosed in the specification itself in a way that one skilled in the art will understand what structure will perform the recited function." MPEP 2181 (II); citing Amtel Corp. v. Information Storage Devices, Inc., 198 F.3d 1374, 1381 (Fed. Cir. 1999). In considering whether there is 35 U.S.C. 112, first paragraph support for a claim element, the Examiner must consider

the summary, detailed description, claims, abstract, and drawings of the original disclosure. MPEP 2181 (IV); In re Mott, 539 F.2d 1291, 1299 (CCPA 1976) (claims); In re Anderson, 471 F.2d 1237, 1240 (CCPA 1973) (claims and abstract); In re Armbruster, 512 F.2d 676 (CCPA 1975) (abstract); Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1564 (Fed. Cir. 1991) (drawings); In re Wolfensperger, 302 F.2d 950, 955-57 (CCPA 1962) (drawings).

The Office acknowledges that dexamethasone is a disclosed means for reducing IL-12p40 production by dendritic cells. Office Action mailed July 26, 2005 at Page 5. The specification, in Example 3, indicates that dexamethasone reduces IL-12p40 production by a dendritic cell. Id., at [0034]. The purpose of Example 3 was to "investigate() whether DEX affected IL-12 production by DC stimulated through CD40." Id., at [0034]. Accordingly, it was shown that "CD40 triggering of DEX-treated DC resulted in a dramatically reduced IL-12p40 production (up to 100 fold)." Id., at [0034]. Also, the specification states, "DEX profoundly affect(ed) the CD40-dependent maturation of human monocyte-derived DC... by causing these cells to secrete the anti-inflammatory mediator IL-10 instead of the Th1 stimulatory cytokine IL-12." Id. at [0005]. Further support for identification of DEX as the structure performing the function of reducing IL-12p40 production is found in Fig. 4 of the drawings. Fig. 4 clearly shows the results of Applicants' experiment, wherein pretreatment of cultured DC with DEX reduced IL-12p40 production after activation with a CD8-CD40L fusion protein. Fig. 4 is described in the specification as "Pretreatment with DEX alters the cytokine secretion profile of CD40-triggered DC." Id., at [0012-13]. This description directs those skilled in the art to Fig. 4, where reduction of IL-12p40 production by DEX is disclosed in quantitative detail. Any of the content described above would be sufficient to disclose DEX to one skilled in the art as the structure for performing the function of reducing IL-12p40 production by a dendritic cell. Together, they unmistakably inform a person having ordinary skill in the art that the intended structure is DEX.

Because claim 1 is drawn to a means for performing the function of reducing IL-12p40 production in a dendritic cell, and the Application identifies dexamethasone as a structure performing that function in a clearly identifiable manner, the Applicants respectfully submit that claim 1 satisfies the written description requirement of 35 U.S.C. 112, first paragraph, as applied to a means-plus-function claim, and is in condition for allowance. Applicants therefore respectfully request the rejection of claim 1 be withdrawn.

B. Claim 1 particularly points out and distinctly claims the subject matter which Applicants regard as the invention.

Alleged basis of rejection

In the Office Action of October 9, 2007, the Examiner rejected claim 1 as allegedly insufficient under the clear claiming requirement of 35 U.S.C. § 112, second paragraph. The Examiner alleged, "[r]egarding the means-plus-function limitations recited in (claim 1), i.e., 'means for reducing IL-12p40 production by said dendritic cell', there does not appear to be any structure in the specification corresponding to these means-plus-function limitations in the claims." 10/09/07 Office Action, at page 5, lines 24-26.

Claim 1 satisfies the statutory requirement for clear claiming, because one skilled in the art could clearly identify dexamethasone as a structure described in the specification for performing the function of reducing IL-12p40 production by dendritic cells.

The Office has issued guidelines in the MPEP for ascertaining compliance with 35 U.S.C. § 112 ¶ 2 when 35 U.S.C. § 112 ¶ 6 is invoked. As a preliminary matter, "[i]f the corresponding structure, material or acts are described in the specification in specific terms (e.g., an emitter-coupled voltage comparator) and one skilled in the art could identify the structure, material or acts from that description, then the requirements of 35 U.S.C. 112, second and sixth paragraphs and (sic) are satisfied." MPEP 2181 (III)A; Atmel Corp. v. Information Storage Devices Inc., 198 F.3d 1374, 1382 (Fed. Cir. 1999). Furthermore, "unless the means-plus-function language is itself unclear, a claim limitation written in means-plus-function language meets the definiteness requirement in 35 U.S.C. 112, second paragraph, so long as the specification meets the written description requirement in 35 U.S.C. 112, first paragraph." MPEP 2181 (II); In re Noll, 545 F.2d 141, 149 (CCPA 1976).

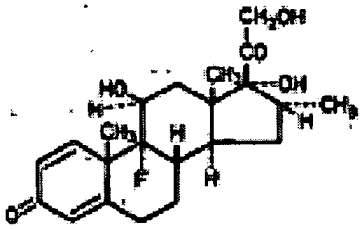
Given Applicants' demonstration (supra) that they have satisfied the written description requirement of 35 U.S.C. § 112 ¶ 1 by identifying DEX as a structure corresponding to the function of reducing IL-12p40 production by a dendritic cell, it follows through application of the rule of In re Noll and the examination guidelines of the MPEP that the element of claim 1 at

issue meets the clear claiming requirement of 35 U.S.C. § 112 ¶ 2.

Dexamethasone is a specific term identifying a structure corresponding to the means-plus-function element of claim 1. Dexamethasone is a known compound in the art with a defined chemical structure. It unambiguously refers to:

Dexamethasone

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16α)-, (*Various M/rs*)



Gennaro, A.Pharmaceutical Sciences (1985) (of record).

One skilled in the art could easily identify DEX as a structure corresponding to the means of the "means-plus-function" element. Applicants used DEX in their disclosed experiments to "reduce(e) IL-12p40 production by (cultured) dendritic cells." The results of these experiments were quantitatively depicted in Fig. 4. Furthermore, the use of DEX to reduce IL-12p40 production by cultured dendritic cells was described in detail in Example 3 of the specification. The specification elsewhere references the results of these experiments, and clearly identifies their value to the invention as demonstrating the reduction of IL-12p40 production in cultured DC by DEX. e.g. at ¶¶ [0005], [0012-13]. The Examiner agrees that Dex is a particular material that has been adequately linked to the function recited. Office Action 7/26/05, p. 5. There can be no doubt that one of skill in the art would identify the specific chemical compound, DEX, as a structure which reduces IL-12p40 production in DC after even a cursory reading of the Applicants' disclosure.

Because claim 1 satisfies the written description requirement in clear means-plus-function claim language, the Applicants respectfully submit that claim 1 satisfies the clear claiming requirement of 35 U.S.C. § 112, second paragraph, and is in condition for allowance. Applicants

therefore respectfully request the rejection of claim 1 be withdrawn.

C. Claims 40-49, 51-53, 55, 56, 58, 59, 61-63, and 65-68 comply with the enablement requirement of 35 U.S.C. § 112, first paragraph.

Alleged basis of rejection

Claims 40-49, 51-53, 55, 56, 58, 59, 61-63, and 65-68 stand rejected for allegedly failing to meet the enablement requirements of 35 U.S.C. § 112 ¶ 1, because "the specification, while being enabling for a method for preparing a loaded, activated dendritic cell or a composition comprising same by treating the cells with DEX, does not reasonably provide enablement for a method for preparing a loaded, activated dendritic cell or a composition comprising same by treating the cells with glucocorticoids in general... [D]isclosure of the species DEX is not representative of a general recitation of the genus glucocorticoids." Office Action 10/09/07, p. 4-5.

Claims at issue

Independent claim 40 recites, "a method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host against an antigen, said method comprising: culturing peripheral blood monocytes from said host to differentiate into dendritic cells; activating said dendritic cells with a glucocorticoid capable of activating a glucocorticoid receptor; bringing said dendritic cells into contact with an antigen against which said T-cell response is to be reduced; and forming a pharmaceutical composition comprising said loaded, activated dendritic cells." Claims 41-49 depend from claim 40.

Independent claim 51 recites, "a method for obtaining a dendritic cell capable of tolerizing a T-cell for an antigen, comprising: providing said dendritic cell with a substance capable of activating a glucocorticoid receptor; activating said dendritic cell; and providing said dendritic cell with said antigen; wherein said dendritic cell is capable of tolerizing a T-cell for said antigen." Claims 52-53, and 55 depend from claim 51.

Independent claim 56 recites, "a method for preparing an isolated dendritic cell, said method comprising: isolating peripheral blood monocytes from a subject; culturing the peripheral blood monocytes to differentiate into dendritic cells; activating the dendritic cells with a glucocorticoid; loading the dendritic cells with an antigen; and isolating said loaded, activated dendritic cells." Claims 58-59, and 61-63 depend from claim 56. Claims 61-62 depend from claim 56, but also depend from allowed dependent claim 60, which recites "the glucocorticoid is dexamethasone."

Independent claim 65 recites, "a method for preparing a dendritic cell for tolerizing a T-cell in a graft or transplant recipient, said method comprising: culturing peripheral blood monocytes from said graft or transplant recipient to differentiate into dendritic cells; activating said dendritic cells; and loading said dendritic cells with an antigen against which said T-cell is to be tolerized."

Independent claims 51 and 65 do not contain any reference to the genus, glucocorticoids. Thus, the rejection of claims 51 and 65 is improper, because the basis of the rejection does not apply to these claims

Claims 40-49, 51-53, 55, 56, 58, 59, 61-63, and 65-68 are fully enabled by the Applicants' disclosure, because use of the genus, glucocorticoid, is enabled by description of the representative species, dexamethasone. The Examiner has offered no reason under which claims that do not refer to the genus, glucocorticoid, are not enabled, and even supposing such a reason, those skilled in the art are given more than adequate guidance by the Application to practice the full scope of the claimed methods in light of the advanced state of the prior art and the high level of skill of those in the art.

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention. 35 U.S.C. § 112, first paragraph.

A claim is enabled under 35 U.S.C. § 112 ¶ 1 if an application, when filed, contains

sufficient information regarding the subject matter of the claim as to enable one skilled in the pertinent art to make and use the claimed invention without undue experimentation. MPEP 2164.01; Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916); In re Wands, 858 F.2d 731, 737 (Fed. Cir. 1988). The disclosure of one species in a claimed genus is sufficient to enable the claim if its practice does not require undue experimentation. Amgen Inc. v. Hoechst Marion Roussel, Inc., 314 F.3d 1313, 1338 (Fed. Cir. 2003) ("When the undisputed facts establish that those skilled in the art could rely on the specification to practice the full scope of the invention without undue experimentation, the invention is enabled even where, as here, only one species is actually disclosed.").

> The presence of only one working example should never be the sole reason for rejecting claims as being broader than the enabling disclosure, even though it is a factor to be considered along with all the other factors. To make a valid rejection, one must evaluate all the facts and evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims... For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation. MPEP 2164.02

Whether experimentation is undue is analyzed in consideration of the "Wands factors:" (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. MPEP 2164.01(a); Wands, 858 F.2d at 737. "The mere fact that experimentation may have been difficult and time consuming does not mandate a conclusion that such experimentation would have been considered to be 'undue' in the art." Falko-Gunter Falkner v. Inglis, 448 F.3d 1357, 1365 (Fed. Cir. 2006). Extensive experimentation is not undue when the experimentation is routine, and the techniques to perform the experimentation are well known to those skilled in the

art. Ex parte Kuben, 83 USPQ.2d 1410 (BPAI 2007); see also Johns Hopkins Univ. v. Cellpro, Inc., 152 F.3d 1342, 1360 (Fed. Cir. 1998). The examiner's analysis of enablement must consider all of the evidence related to each of the "Wands factors," and any conclusion of nonenablement must be based on the evidence as a whole. MPEP 2164.01(a); Wands, 858 F.2d at 737, 740.

I. Use of the genus, glucocorticoid, within the scope of the Applicants' claims 40-49, 56, 58-59, 63, and 66 is enabled by data in the disclosure using the species, dexamethasone.

The Applicants note that currently rejected claims 61-62, and 67 recite the species, dexamethasone. The Examiner has not argued the claimed methods are not enabled with respect to dexamethasone. In the Office Action of 10/09/07, the Examiner stated, "the specification (is) enabling for a method of preparing a loaded, activated dendritic cell or a composition comprising same by treating the cells with DEX." Therefore, the Applicants believe claims 61-62, and 67 would be allowable if rewritten in dependent form, and respectfully request clarification.

Applicants' use of dexamethasone is enabling with respect to the genus of glucocorticoids, in general, because glucocorticoids are a comprehensively studied family of structurally related compounds with known differences that are the basis of their widespread clinical utility. Those skilled in the art routinely extrapolate the glucocorticoid effects of dexamethasone to those of other glucocorticoids according to empirical kinetic data. These facts are found in the record under a review of the Wands factors.

(A) The breadth of the claims: Claims 40-49, 56, 58-59, 63, and 66 are narrowly drawn to methods that include a step of activating cultured dendritic cells with a glucocorticoid.

Dependent claims 41-49 include the limitations of independent claim 40, which requires "activating said dendritic cells with a glucocorticoid capable of activating a glucocorticoid receptor," wherein said dendritic cells are obtained by "culturing peripheral blood monocytes from (the) host to differentiate into dendritic cells." Therefore, the dendritic cells that are the subject of glucocorticoid treatment in claims 40-49 are necessarily in culture. Likewise, independent claim 56 requires "culturing the peripheral blood monocytes to differentiate into dendritic cells," and claims 58-59, and 63 depend from claim 56. Dependent claim 66 similarly requires "culturing peripheral blood monocytes... to differentiate into dendritic cells" via its dependence on indendent claim 65. The steps of applying glucocorticoids to dendritic cells in

claims 40-49, 56, 58-59, 63, and 66, then, must necessarily be performed on cultured cells. This requirement dramatically decreases the amount of experimentation required to practice the claims, as the greatest part of the unpredictability of glucocorticoids arises from their systemic use.

The enabling character of DEX for the genus of glucocorticoids is particularly demonstrated where those of the Applicants' claims that recite "glucocorticoid" are directed to the use of glucocorticoids in cell culture, and the effects of glucocorticoids on the whole organism are not a consideration. As has long been appreciated, "[w]hen a compound or composition claim is limited by a particular use, enablement of that claim should be evaluated based on that limitation." MPEP 2164.01(c); In re Vaeck, 947 F.2d 488, 495 (Fed. Cir. 1991). The Applicants' invention is not concerned with the many other effects of glucocorticoids; it is directed to the use of glucocorticoids to activate cultured dendritic cells through the glucocorticoid receptor.

(B) The nature of the invention: Claims 40-49, 56, 58-59, 63, and 66 describe methods within the field of clinical immunology.

The preamble of independent claim 40 recites that it is drawn to "[a] method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host against an antigen." Independent claim 56 is "[a] method for preparing an isolated dendritic cell." And, independent claim 65 is "[a] method for preparing a dendritic cell for tolerizing a T-cell in a graft or transplant recipient." The T-cell response is an integral component of a subject's immune response to an antigen. Alberts, et al., of record. "[R]educing an unwanted T-cell response in a host against an antigen" is the subject of claims 40-49. Dendritic cells are antigen-presenting cells that also are a locus of immune response regulation. All of claims 40-49, 56, 58-59, 63, and 66 have the nature of being within the field of clinical immunology. Alberts, et al. Therefore, those employing claims 56, 58-59, and 63 will be those skilled in manipulating cells of the immune system, and who are familiar with the growth and differentiation requirements of those cells.

(C) The state of the prior art: The prior art is in an advanced state wherein glucocorticoids are used interchangeably in appropriate circumstances according to clinical indications.

Glucocorticoids are routinely used in a variety of clinical settings where regulation of an immune response is desirable. [0004] ("The current treatment of (autoimmune disease, allograft rejection and graft versus host disease) largely relies on the administration of glucocorticoids..., which exert potent anti-inflammatory and immunosuppressive effects."). Because glucocorticoids are classified according to biological actions that "lend themselves to quantitative measurement," and "the use of corticosteroids and their congeners in disease is largely empirical," clinical use of glucocorticoids is enabled by following identified therapeutic principles. Goodman et al., "Adrenocorticotropic Hormone", in Goodman and Gilman's The Pharmacological Basis of Therapeutics (1985), of record, 1466, 1479. Moreover, "[e]ffective clinical use of the corticosteroids has become possible because of their isolation, elucidation of structure, and economical synthesis." Id. At 1460. These facts are well-known in the art, and have allowed those skilled in the art to compile reference tables, such as Id., Table 63-3, wherein the function of dexamethasone is quatitatively related to 12 other corticosteroids. Id. At 1475. The state of the prior art, then, is that clinical immunologists are able to use glucocorticoids effectively, because the relative effects of members of this genus have been characterized quantitatively, and individual members are able to be selected according to recognized therapeutic principles.

(D) The level of one of ordinary skill: The level of ordinary skill in clinical immunology is high. Ex parte Kubin and Goodwin, 83 USPQ.2d 1410, 1416 (Bd.Pat.App. & Int. 2007) (molecular biology).

At least as early as 1985, those skilled in the relevant art were able to use the well-characterized pharmacological profiles of different glucocorticoids to select the individual glucocorticoid most appropriate for clinical treatment of a subject, and to use the selected glucocorticoid for treatment. Goodman et al..

(E) The level of predictability in the art: The effects of glucocorticoids in different systems are comprehensively defined, and greatly simplified by use on dendritic cells in culture.

While different glucocorticoids do exhibit slightly different pharmacological profiles, their clinical use, and more particulary, their use on cultured cells, is extremely predictable. As represented in Table 63-3 of Goodman et al., the relative potencies and equivalent therapeutic doses of different glucocorticoids have been empirically measured. These results are widely

available to those skilled in the art. Also, the relative mineralcorticoid activities of different glucocorticoids, which may in some instances be undesirable, are known. Stanford HOPES Project. This high level of knowledge with respect to the similarities and differences of glucocorticoids not only removes the substantial unpredictability normally present in pharmacology, but is a benefit to the practitioner in that it enables him/her to make an informed decision of which glucocorticoid among a variety of distinguishable alternatives to use in a particular subject upon consideration of known principles. Goodman et al. at 1479.

The use of glucocorticoids in cultured cells is even more predictable. In cultured macrophages, "[t]he potency ratios of dexamethasone, prednisolone, and hydrocortisone are in reasonable agreement with their clinical anti-inflammatory activities." Bray, of record, at 640; see also Bray, Table 1, Group 2. Bray, et al., identified cortisone acetate as the only glucocorticoid that did not exhibit activity in culture in agreement with its clinical activity, but that result was easily explained by the failure of cultured macrophages to convert cortisone to hydrocortisone, which is its active derivative. Id. at 640. By using glucocorticoids on cultured dendritic cells, those skilled in the art avoid complication from the compounds' different mineralcorticoid activities, because those activities are attributable to cells of the distal tubules of the kidney and other tissues. Goodman et al. at 1468. Therefore, the high level of predictability in glucocorticoid use that allows this class of drugs to be used effectively in the clinic is even greater when their use is simplified by restriction to cultured cells.

(F) The amount of direction provided by the inventor: Applicants have provided ample direction by describing the use of a representative glucocorticoid according to the methods of claims 40-49, 56, 58-59, 63, and 66 in rigorous detail.

Applicants have directed those skilled in art to employ the claimed methods by use of a representative glucocorticoid, dexamethasone. Example 4 of the application describes the use of dexamethasone to produce antigen-presenting cells that are poor inducers of T cell responses and exert hyporesponsive effects. The steps of independent claim 56 are each taught in the disclosed "Materials and Methods," to wit: (1) isolating peripheral blood monocytes from a subject, [0036]; (2) culturing the peripheral blood monocytes to differentiate into dendritic cells, [0036]; (3) activating the dendritic cells with a glucocorticoid, [0037-38]; and (4) loading the dendritic cells with an antigen, [0040]; where (5) isolating said loaded, activated dendritic cells is well-

known to those skilled in the art of clinical immunology. Neither of claims 58-59 adds an additional step to this method. Claim 63 depends on claim 56, and requires "incubating the dendritic cells with a substance selected from a group consisting of a CD8-40L fusion protein, a trimeric form of CD40L consisting of CD40L molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, and cells that express CD40L." Detailed direction for this additional requirement can be found in Example 1 and Example 4 of the application. [0030, 0035]. The methods of claims 40-49, 58-59, 63, and 66 do not comprise any other steps beyond those described in the application that are not well within the grasp of those skilled in the art. These methods all are drawn to the preparation and use of dendritic cells according to the steps described for claim 56 above, what is described in detail in the application, for example between [0030] and [0044], and the high level of knowledge and skill of those in the art.

Applicants used the glucocorticoid, dexamethasone, in the experiments disclosed in their application. Dexamethasone is a representative glucocorticoid that is understood by those skilled in the art to reflect the activity of glucocorticoids in general. All glucocorticoids act through the glucocorticoid receptor, as assayed by inhibition by the glucocorticoid receptor antagonist, RU486. [0030]; Cronstein, et al., at 9992. Furthermore, the existence of comparative data, such as that contained in Table 63-3 of Goodman et al., makes the extrapolation of data obtained with dexamethasone to other glucocorticoids a simple matter.

The record overwhelmingly reflects the fact that those skilled in the art refer to "dexamethasone" and "glucocorticoids" interchangeably, in recognition of the fact that dexamethasone is a representative drug in this highly predictable class. In Cronstein, et al., the authors "determined whether *corticosteroids* suppress inflammation by inhibiting endothelial expression of adhesion molecules for neutrophils" with dexamethasone. Id. at 9991, of record. The Stanford HOPES project similarly describes research conducted with dexamethasone as yielding results that can be extended to other glucocorticoids: The HOPES project describes the import of several investigators' work for those skilled in the art.: In Newton, et al., "an experiment to try to explain the mechanism by which the glucocorticoid dexamethasone suppresses the production of mediators involved in inflammation" yielded results "indicat(ing) that *glucocorticoids such as dexamethasone* exert their anti-inflammatory effects through a variety of mechanisms." HOPES project. at 3-4; In Diamond, et al., the researchers concluded

that "(aggregation of expanded polyglutamine proteins) can be manipulated by *glucocorticoid*-controlled gene expression" after finding that "the addition of *dexamethasone* to (cells) expressing (Huntington's Disease) reduced the aggregation of the altered huntington protein... (and) *dexamethasone* administration to cells expressing spinobulbar muscular atrophy showed decreased androgen receptor (AR) aggregation." HOPES project. at 5-6. Therefore, the HOPES project's characterization of the art indicates that it was customary in the art for dexamethasone to be used as a representative glucocorticoid with respect to their physiological actions. Again, dexamethasone is further representative of the actions of glucocorticoids acting in cultured cells through the glucocorticoid receptor, as determined by RU486 competition.

(G) The existence of working examples: Applicants have disclosed the use of dexamethasone as a working example of the methods of claims 40-49, 56, 58-59, 63, and 66.

The Office has agreed the Applicants' disclosure is enabling with respect to dexamethasone. Office Action of 10/09/2007, at 4 ("[T]he specification (is) enabling for a method of preparing a loaded, activated dendritic cell or a composition comprising same by treating the cells with Dex"). Claims 40-49 claim methods for preparing pharmaceutical compositions comprising activated dendritic cells. Also, claims 56, 58-59, 63, and 66 claim methods for preparing loaded, activated dendritic cells. Therefore, the fact that the Applicants have provided a detailed working example of their invention is not in dispute.

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure: Using the methods of claims 40-49, 56, 58-59, 63, and 66 requires only routine experimentation, guided by the content of the disclosure and the comprehensive information available on the pharmacological profiles of all glucocorticoids.

"[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." MPEP 2164.06, quoting In re Colianni, 561 F.2d 220, 224 (CCPA 1977) and Wands, 858 F.2d at 737. Furthermore, "[i]n the chemical arts, the guidance and ease in carrying out an assay to achieve the claimed objectives may be an issue to be considered in determining the quantity of experimentation needed... Time and difficulty of

experiments are not determinative if they are merely routine." MPEP 2164.06.

In Goodman et al., the first therapeutic principle of using glucocorticoids to treat disease is stated as "(1) for any disease, in any patient, the appropriate dose to achieve a given therapeutic effect must be determined by trial and error and must be reevaluated from time to time as the stage and the activity of the disease alter." Id. at 1479. This reference shows, therefore, that time-consuming experiments using different doses of glucocorticoids are routine in the art of clinical immunology. Such periodic measurement of the dose-dependence of glucocorticoid efficacy is, in fact, a necessary condition of glucocorticoid therapy. The Applicants' invention requires considerably less experimentation than this routine amount. Because the Applicants' claims are directed to application of glucocorticoids to dendritic cells in culture, the dose-dependence of the dendritic cells' response to a particular glucocorticoid is far more easily ascertainable. Figure 3 of Bray, et al., shows the dose-response relations of five different glucocorticoids in cultured cells. Id. at 639. Unlike the routine periodic testing required for use of glucocorticoids systemically to treat disease, the responsiveness of one type of cultured cell to a particular glucocorticoid need only be determined once.

Claims 40-49, 56, 58-59, 63, and 66 are enabled under 35 U.S.C. § 112 ¶ 1, because the Applicants' application, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention without undue experimentation. MPEP 2164.01; Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916); In re Wands, 858 F.2d 731, 737 (Fed. Cir. 1988). The preceding review of the Wands factors in this case shows that those highly skilled individuals in the art of clinical immunology are familiar with comprehensive quantitative comparisons of the effect of different glucocorticoids in clinical use. Dexamethasone is used by those skilled in the art as a representative member of the class of glucocorticoids, because its effects are able to be extrapolated to other glucocorticoids through use of the available comparative data. Furthermore, those skilled in the art perform routine experimentation during glucocorticoid therapy to determine which glucocorticoid is preferred for treatment, and whether its use should be continued in a patient. Importantly, the requirement that the relevant steps of the Applicants' claimed invention take place in cultured cells substantially reduces the quantity and difficulty of even this routine experimentation that is required. Because the Applicants' invention only

requires the use of glucocorticoids on a single cell type, dendritic cells, in culture, systemic mineralcorticoid effects do not need to be considered, and a single dose-response experiment with a glucocorticoid is sufficient for one skilled in the art to use that glucocorticoid in the claimed methods. The readily available comparative data on the effects of different glucocorticoids, and the Applicants' detailed direction and working example with dexamethasone, allow those skilled in the art to use the claimed methods without undue experimentation. Consequently, use of the genus, glucocorticoids, in the claimed methods is enabled by use of the single, representative species, dexamethasone.

The evidence cited by the Examiner supports a conclusion of enablement.

In view of the strong case of enablement made out above, the reasons proffered by the Examiner to support the pending enablement rejection are insufficient to sustain the rejection. "Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation." MPEP 2164.02. The Examiner has cited two references to support his contention that "disclosure of the species (dexamethasone) is not representative of a general recitation of the species glucocorticoids." Office Action of 10/09/07, at 4. In the first instance, the Examiner states that "Stanford's HOPES project (of record), teaches that not all glucocorticoids comprise the same biological activities. (Dexamethasone) is described as having more anti-inflammatory activity than prednisone or hydrocortisone, and hydrocortisone is described as having more undesirable activity than either (dexamethasone) or prednisone." For further support, the Examiner goes on to recite that "(the HOPES project) echoes what has been well known in the art for some time, i.e., that glucocorticoids comprise different biological activities in different contexts." Bray, et al. Both of these are tortured interpretations of the cited material. In fact, both of the cited references stand for the proposition that dexamethasone is a representative glucocorticoid, from which the activities of other glucocorticoids on cultured dendritic cells may be extrapolated without undue experimentation.

The Stanford HOPES project broadly distinguishes between the relative amounts of glucocorticoid and mineralcorticoid activity exhibited by different glucocorticoids. Although a

specific citation to support the Examiner's contention was absent from the Office Action of 10/09/07, the Applicants believe the Examiner is referring to the statements on page 3 of the reference. The statements are as follows:

> In addition to the difficulty of separating the metabolic and anti-inflammatory effects of glucocorticoids, most synthetic drugs often referred to as glucocorticoids are actually synthetic corticosteroids. These synthetic drugs have both mineralcorticoid and glucocorticoid activity. However, in a particular compound, one type of activity will predominate over the other. Synthesis of pure glucocorticoid drugs has so far been elusive.
>
> Commonly prescribed steroid drugs:
>
> Prednisone and Prednisolone – Most commonly used glucocorticoid because of its high glucocorticoid activity. Prednisone is transformed by the liver into prednisolone. Prednisolone may be administered in tablet form or produced by the body from prednisone. These medications are often considered to be interchangeable.
>
> Dexamethasone – Has a particularly high glucocorticoid activity and low mineralcorticoid activity and can therefore be used in high doses. Often used to reduce nerve swelling following neurotrauma and neurosurgery.
>
> Hydrocortisone – Has much more mineralcorticoid activity than Prednisone and is therefore not suitable for long-term use internally. Externally, it is used extensively as a cream or lotion for skin conditions such as rashes or itches. Id. at 3.

Reliance on this reference by the Examiner is inapposite. In fact, the above passage does not contradict the teaching of the Stanford HOPES reference as a whole: that all glucocorticoids exhibit glucocorticoid activity that is comparable to other glucocorticoids according to known, empirically-determined relationships. Furthermore, the undesirable mineralcorticoid activities compared in the above passage are not applicable to the Applicants' methods, which are necessarily performed in cell culture. In short, the Examiner's objection that "not all glucocorticoids comprise the same biological activities" is without merit, because the Stanford HOPES reference, as well as the other references in the record, teaches that all glucocorticoids differ only in the relative magnitudes of their glucocorticoid activities, and those relative magnitudes have been empirically determined and are known to those skilled in the art.

Moreover, the "other biological activities" attributable to glucocorticoids are irrelevant to the Applicants' claimed methods, because those methods are performed on dendritic cells in culture. As a further matter, the Applicants point out that their method claims are directed to preparing isolated dendritic cells, and pharmaceutical compositions comprising the same. Other biological activities are not relevant to the examination of these claims; it is obviously possible to practice the claimed invention as part of a broader therapeutic regime, or within a background of unrelated glucocorticoid activity.

The Examiner's reliance on the Bray, et al. reference is also misplaced. According to the Examiner, Bray teaches that "glucocorticoids comprise different biological activities in different contexts." Bray, et al. describe their results in the following way: "Seven anti-inflammatory glucocorticosteroid preparations inhibited prostaglandin production in a dose-related manner. The relative potencies of dexamethasone, prednisolone and hydrocortisone were consistent with clinical anti-inflammatory ranking. Cortisone, however, failed to inhibit macrophage prostaglandin production." Bray, et al. at 635 (Item 3). The data of Bray, et al. are exactly the sort of commonly-available information that renders dexamethasone an enabling representative example of glucocorticoids. Figure 3 and Table 1 of Bray, et al. summarize their data; empirical dose response relations and kinetic parameters are listed for seven glucocorticoids. With these data, those skilled in the art can easily convert an effective concentration of dexamethasone to an equally effective concentration of other glucocorticoids. The Examiner's statement that this reference teaches "different biological activities" for different glucocorticoids is incorrect. In fact, it teaches that different glucocorticoids are effective at different concentrations for the same biological activity. Moreover, Bray, et al. state that the relative potencies of the glucocorticoids they tested was "consistent with their clinical ranking." Id. at 635. The authors thereby teach that the vast amount of data with respect to the relative clinical efficacies of different glucocorticoids can be extrapolated to their use with cultured cells. The authors' discovery that cortisone failed to exhibit anti-inflammatory activity does not alter this conclusion, as they are able to readily attribute the ineffectiveness of cortisone to the failure of macrophages to convert cortisone to what is in fact the biologically active compound, hydrocortisone. Therefore, Bray, et al. is completely consistent with the fact that glucocorticoids are a well-described family of structurally related compounds, and that the Applicants' detailed example of using

dexamethasone in their claimed methods is enabling to those skilled in the art who have access to the comprehensive comparative data available for this class of compounds that was available at the time the invention was made.

It is also true that, even were this comparative data absent, practice of the claimed methods with glucocorticoids other than dexamethasone would not require undue experimentation. All glucocorticoids share activity through the glucocorticoid receptor, which can be determined conclusively by RU486 inhibition. Furthermore, dose response relations that establish the effective amount of a glucocorticoid in cultured cells are easily obtained according to simple and standard experiments that are well known in the art, of which Figure 3 of Bray, et al. is an example. Such an experiment would need only be performed once for any specific glucocorticoid on cultured dendritic cells.

II. Applicants' claims that do not recite the genus, glucocorticoid, are also enabled by the disclosure.

The reasoning offered by the Examiner in the Office Action of 10/09/07 can only serve to allege invalidity of claims 40-49, 56, 58-59, 61-63, and 66-67. The Examiner's rejection cannot apply to claims 51-53, 55, 65, or 68, because none of these claims contain any recitation of the genus, glucocorticoids. According to In re Bowen, 492 F.2d 859, 862-63, 181 USPQ 48, 51 (CCPA 1974), "the minimal requirement to sustain an enablement rejection is for the examiner to give reasons for the uncertainty of the enablement. This standard is applicable even when there is no evidence in the record of operability without undue experimentation beyond the disclosed embodiments." See also In re Brana, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (citing In re Bundy, 642 F.2d 430, 433, 209 USPQ 48, 51 (CCPA 1981)). As the Examiner has given no reasons for the uncertainty of the enablement of claims 51-53, 55, 58-59, 65, or 68, the Applicants believe those claims are enabled. Nevertheless, without specific arguments to address, the Applicants will support the enablement of these claims from the record.

"A patent need not teach, and preferably omits, what is well known in the art." MPEP 2164.01; In re Buchner, 929 F.2d 660, 661 (Fed. Cir. 1991); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and

Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 1463 (Fed. Cir. 1984). "(A) process () for dealing with a large class of substances and the range of treatment within the terms of the claims, while leaving something to the skill of persons applying the invention is clearly sufficiently definite to guide those skilled in the art to its successful application." In re Angstadt, 537 F.2d 498, 502-4 (Cust. & Pat.App. 1976) (citing In re Mercier, 515 F.2d 1161, 1167-68 (Cust. & Pat.App. 1975), In re Fisher, 427 F.2d 833, 839 (Cust. & Pat.App. 1970)); MPEP 2164.06.

The steps of "providing (a) dendritic cell with a substance capable of activating a glucocorticoid receptor," and "activating (cultured) dendritic cells", are routine for those skilled in clinical immunology. Standard and routine testing confirms that a particular substance activates a glucocorticoid receptor. Furthermore, assaying the activation of dendritic cells is commonplace. Abundant evidence in the record supports these assertions. The inclusion of either of these steps does not render claims 51-53, 55, 65, and 68 nonenabled when the "Wands factors" are considered to determine whether the amount of experimentation necessary to practice the claimed methods is undue. MPEP 2164.01(a); Wands, 858 F.2d at 737, 740.

(A) The breadth of the claims: Claims 51-53, 55, 65, and 68 are drawn to methods of "obtaining" or "preparing" a cultured dendritic cell capable of tolerizing a T-cell.

As was explained previously, it is important that the methods of claims 65 and 68 are necessarily performed in culture. Because "[w]hen a compound or composition claim is limited by a particular use, enablement of that claim should be evaluated based on that limitation," the relevant question with respect to enablement of claims 65 and 68 is whether the step of "activating () dendritic cells" is enabled when those cells are in culture. MPEP 2164.01(c); In re Vaeck, 947 F.2d 488, 495 (Fed. Cir. 1991). The activation of cultured dendritic cells is routine for those skilled in the art, and successful completion of this step does not require undue experimentation. Claims 51-53, and 55 require the steps of "providing (a) dendritic cell with a substance capable of activating a glucocorticoid receptor" and "activating said dendritic cell." Determining whether a particular substance is capable of activating a particular receptor is routine when an antagonist of the receptor is available. The Applicants' examples describe the use of RU486 as a glucocorticoid receptor antagonist, for example at [0030] of the Application. This activity of RU486 is well known to those skilled in the art. Furthermore, neither is it the

case that activating a dendritic cell requires undue experimentation.

(B) The nature of the invention: Claims 51-53, 55, 65, and 68 describe methods within the field of clinical immunology.

Claims 51-53, and 55 are methods for "obtaining a dendritic cell capable of tolerizing a T-cell for an antigen." Claims 65 and 68 are methods for "preparing a dendritic cell for tolerizing a T-cell in a graft or transplant recipient to differentiate into dendritic cells." Therefore, these claims are within the field of clinical immunology.

(C) The state of the prior art: The prior art is replete with information and instruction regarding glucocorticoid receptor pharmacology and dendritic cell culture.

RU486 is a glucocorticoid receptor antagonist that is routinely used to determine whether a compound binds to a glucocorticoid receptor. For example, Cronstein, et al. (of record) teaches that the effects of RU486 can be used to "determine whether the (effect of) dexamethasone was receptor-mediated." Id. at 9992. Cronstein, et al. also suggest an alternative glucocorticoid receptor antagonist for this purpose, tetrahydrocortisol. Id. at 9992. The Applicants describe the use of RU486 at, for example, [0030] of the Application. Consequently, the prior art shows that glucocorticoid receptor activation was easily determined by use of at least two receptor antagonists prior to the Applicants' disclosure, and that those antagonists are sufficient for use in the simple and routine experimentation required to determine whether a substance is capable of activating the glucocorticoid receptor in the Applicants' claimed methods.

The process by which dendritic cells are activated during immunity has been the subject of extensive experimentation in recent decades. A minimal description of the understanding to be gleaned from this prior art is described in [0003] of the Application (Background of the Invention): "(Dendritic cell) activation is triggered in inflamed tissues by cytokines such as IL-1 and TNF-a and by bacterial components such as LPS... Upon interaction with antigen-specific T cells, (dendritic cell) activation is further completed through engagement of the receptor-ligand pair CD40-CD40L." (Internal citations omitted). Furthermore, the step of activating dendritic cells with CD40-CD40L is taught in Hancock, et al. (of record; "binding of CD40L to CD40 during cognate T-B interactions provides B cell help." Internal citations omitted). Kampgen, et al. (of record) further describe the activation of Langerhans cells, a type of dendritic cell, by tissue culture.

Activation of dendritic cells in culture as required in, for example, claims 65 and 68, is particularly commonplace. Although the levels of compounds such as IL-1, TNF-a and LPS that can be administered systemically are readily ascertainable by those skilled in the art, such considerations do not even apply to steps performed in culture. Performing the activation of dendritic cells in culture eliminates the need for careful observation and individual subject treatment that accompanies any steps performed in the whole organism.

(D) The level of one of ordinary skill: The level of ordinary skill in clinical immunology is high. Ex parte Kubin and Goodwin, 83 USPQ.2d 1410, 1416 (Bd.Pat.App. & Int. 2007) (molecular biology).

(E) The level of predictability in the art: Substances capable of activating glucocorticoid receptors are well-known, as are techniques for activating dendritic cells, either in culture or otherwise. To overcome the low predictability of unknown substances and techniques requires only some experimentation.

As was described in section (C) above, several excellent glucocorticoid receptor antagonists are well-known in the art, and their use in determining whether a particular compound acts through a glucocorticoid receptor is routine. Also, a variety of standard methods for activating a dendritic cell are known to those with skill in the art. Should one of skill in the art attempt to use other substances to activate a glucocorticoid receptor, or other methods of activating a dendritic cell, beyond those described in the prior art or the Application, only routine experimentation is required to test whether the new substance or method will work in the claimed invention.

However inherently unpredictable immunology may be in general, the existence of simple experimental techniques that assay a unique and targeted function render the measurement of the effectiveness of different ways of accomplishing that function routine. Activated dendritic cells can easily be identified in a sample by immunohistochemistry using specific antibodies such as those utilized in the Hancock, et al. reference. Therefore, in order to identify an adequate method of activating a dendritic cell, one skilled in the art only needs to test the ability of their method to produce dendritic cells that are immunoreactive with known antibodies. Substances capable of activating a glucocorticoid receptor are likewise easy to identify. For example, the prostaglandin E inhibition assay utilized by Bray, et al. in Figures 3 and 4 of their reference can be conducted in

the presence of a glucocorticoid receptor antagonist to determine whether a substance activates that receptor. If the prostaglandin E inhibition is reversed by the antagonist, then the substance activates a glucocorticoid receptor.

(F) The amount of direction provided by the inventor: Applicants' disclosure directs those skilled in the art to substances capable of activating a glucocorticoid receptor, and to successful activation of cultured dendritic cells.

In their Application (Summary of the Invention), the Applicants direct those skilled in the art to glucocorticoids, and dexamethasone in particular, as substances capable of activating a glucocorticoid receptor. ("Importantly, the present invention clearly demonstrates that GC (glucocorticoids) such as DEX (dexamethasone) do not simply suppress (dendritic cell) activation but rather redirect this process towards a distinct functional program;" [0006]). Dexamethasone is identified as a substance that binds to the glucocorticoid receptor by RU486 inhibition. [0030]. In the Background section of the Application, the Applicants list three different ways to activate a dendritic cell: IL-1, TNF-a, and by bacterial components such as LPS. [0003]. These three methods are used in the detailed Examples provided by the Applicants in their Specification.

(G) The existence of working examples: Applicants have described the use of the methods of claims 51-53, 55, 65, and 68, in detailed Examples 1-4. Together the Examples teach (1) isolation of peripheral blood monocytes (Materials and Methods; [0036]); (2) use of a representative glucocorticoid, dexamethasone, to activate a glucocorticoid receptor (Examples 1-4); (3) activation of dendritic cells by CD8-CD40L fusion protein (Example 1; [0030]), (4) loading dendritic cells with an antigen (FITC-BSA; Example 2; [0033]), and (5) use of a dendritic cell produced according to the invention to induce a state of hyporesponsiveness in T cells ("tolerized T-cells;" Example 4; [0035]). These are all the steps required to practice claims 51-53, 55, 65, and 68.

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure: Those skilled in the art can easily determine if a particular substance activates a glucocorticoid receptor, or if a proposed technique activates a cultured dendritic cell, through only routine experimentation.

Applicants reiterate that the Examiner has not provided any reason supporting his

enablement rejection of claims 51-53, 55, 65, and 68, because those claims do not recite the genus, glucocorticoid. And, the Examiner's enablement rejection relies on the Examiner's belief that use of that genus is not enabled by the representative species, dexamethasone. Because the Applicants assert that the full scope of their claims is enabled, and the Examiner has not provided a reason under which enablement can be questioned, the Applicants respectfully submit that claims 51-53, 55, 65, and 68 are in condition for allowance. In re Bowen, 492 F.2d at 862-63. Anticipating the Examiner's arguments to the extent possible, the Applicants believe they have shown that, under the Wands factor analysis, practice of the full scope of these claims does not require undue experimentation.

A claim is enabled, even though it requires some experimentation, if that experimentation is not undue. "[A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.'" MPEP 2164.06, quoting In re Colianni, 561 F.2d 220, 224 (CCPA 1977) and Wands, 858 F.2d at 737. And, "[i]n the chemical arts, the guidance and ease in carrying out an assay to achieve the claimed objectives may be an issue to be considered in determining the quantity of experimentation needed... Time and difficulty of experiments are not determinative if they are merely routine." MPEP 2164.06.

The level of skill in the art of clinical immunology is high, and the prior art teaches a genus of compounds that are substances capable of activating a glucocorticoid receptor (glucocorticoids), and many methods for activating dendritic cells. The prior art also teaches simple and routine assays for determining whether a substance is capable of activating a glucocorticoid receptor (receptor antagonist inhibition) and for measuring the successful activation of dendritic cells (immunohistochemistry). Those with the high level of skill that is the standard in the art perform these assays with ease. Moreover, the Applicants have described in detail the practice of every step of their claimed methods in their Specification. With the guidance, direction, and working examples given in the Application, those skilled in the art can easily use the prior art in combination to duly practice the claimed methods according to their preferred embodiment.

Because the Applicants' detailed example of using a representative glucocorticoid, dexamethasone, provides instruction that is readily analogized across the genus of

glucocorticoids according to the practice and expectations of those skilled in the art, the Applicants respectfully submit that claims 40-49, 51-53, 55, 56, 58, 59, 61-63, and 65-68, are enabled under 35 U.S.C. 112, paragraph 1. Therefore, the Applicants respectfully request the rejections of these claims by withdrawn.

Conclusion

For at least the foregoing reasons, appellants respectfully submit that all pending claims are in condition for immediate allowance. Consequently, appellants respectfully request that the rejection of claim 1 under 35 U.S.C. 112 ¶ 1 for lack of written description and clear claiming, and the rejection of claims 40-49, 51-53, 56, 58, 59, 61-63, and 65-68 under 35 U.S.C. 112 ¶ 1 for lack of enablement, be withdrawn, and the claims be allowed.

(8) CLAIMS APPENDIX

1. A method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host, said method comprising:

culturing peripheral blood monocytes from said host to differentiate into dendritic cells;

activating said dendritic cells with a means for reducing IL-12p40 production by said dendritic cells;

loading said dendritic cells with an antigen against which said T-cell response is to be reduced; and

forming a pharmaceutical composition comprising said loaded, activated dendritic cells for administration to said host.

40. A method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host against an antigen, said method comprising:

culturing peripheral blood monocytes from said host to differentiate into dendritic cells;

activating said dendritic cells with a glucocorticoid capable of activating a glucocorticoid receptor;

bringing said dendritic cells into contact with an antigen against which said T-cell response is to be reduced; and

forming a pharmaceutical composition comprising said loaded, activated dendritic cells.

41. The method according to claim 40, further comprising activating a CD40 receptor on said dendritic cells.

42. The method according to claim 41, wherein activating the CD40 receptor comprises incubating the dendritic cells with a substance selected from the group consisting of a CD8-40L fusion protein, a trimeric form of CD40L consisting of CD40L molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, and cells that express CD40L.

43. The method according to claim 40, wherein bringing said dendritic cells into contact with an antigen comprises incubating said dendritic cells with at least one peptide representing at least one antigen of interest before activating said dendritic cells with said substance capable of activating the glucocorticoid receptor.

44. The method according to claim 40, wherein bringing said dendritic cells into contact with an antigen comprises incubating said dendritic cells with cells containing at least one antigen of interest before activating said dendritic cells with said substance capable of activating the glucocorticoid receptor .

45. The method according to claim 40, wherein bringing said dendritic cells into contact with an antigen against which said T-cell response is to be reduced comprises loading said dendritic cells with at least one synthetic peptide representing at least one antigen of interest after activating said dendritic cells with said substance capable of activating the glucocorticoid receptor .

46. The method according to claim 40, wherein activating said dendritic cells with said substance capable of activating the glucocorticoid receptor comprises activating said dendritic cells such that said dendritic cells secrete interleukin-10.

47. The method according to claim 40, wherein said T-cell is a T-helper cell.

48. The method according to claim 40, wherein bringing said dendritic cells into contact with an antigen comprises incubating said dendritic cells with a cell homogenate containing at least one antigen of interest before activating said dendritic cells with said substance capable of activating the glucocorticoid receptor.

49. The method of claim 40, further comprising incubating the dendritic cells with a substance selected from the group consisting of lipopolysaccharide (LPS) and polyI/C.

51. A method for obtaining a dendritic cell capable of tolerizing a T-cell for an antigen, comprising:

providing said dendritic cell with a substance capable of activating a glucocorticoid receptor;

activating said dendritic cell; and

providing said dendritic cell with said antigen, wherein said dendritic cell is capable of tolerizing a T-cell for said antigen.

52. The method according to claim 51, wherein providing said dendritic cell with the substance capable of activating a glucocorticoid receptor is in vitro.

53. The method according to claim 51, wherein providing said dendritic cell with said substance capable of activating the glucocorticoid receptor comprises providing a precursor of said dendritic cell with said substance capable of activating the glucocorticoid receptor in vitro.

55. The method according to claim 52, wherein said substance capable of activating the glucocorticoid receptor enhances secretion of IL-10 by said dendritic cells.

56. A method for preparing an isolated dendritic cell, said method comprising:

isolating peripheral blood monocytes from a subject;

culturing the peripheral blood monocytes to differentiate into dendritic cells;

activating the dendritic cells with a glucocorticoid;

loading the dendritic cells with an antigen; and

isolating said loaded, activated dendritic cells.

58. The method according to claim 56, wherein loading said dendritic cells with an antigen comprises loading said dendritic cells with an antigen defined by a response of a T-cell.

59. The method according to claim 56, wherein the antigen comprises an allogeneic antigen.

61. The method according to claim 60, wherein loading said dendritic cells with an antigen comprises contacting said dendritic cells with cells derived from a graft or transplant donor.

62. The method according to claim 61, wherein the dendritic cells are derived from the graft or transplant recipient.

63. The method according to claim 56, further comprising incubating the dendritic cells with a substance selected from a group consisting of a CD8-40L fusion protein, a trimeric form of CD40L consisting of CD40L molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, and cells that express CD40L.

65. A method for preparing a dendritic cell for tolerizing a T-cell in a graft or transplant recipient, said method comprising:

culturing peripheral blood monocytes from said graft or transplant recipient to differentiate into dendritic cells;

activating said dendritic cells; and

loading-said dendritic cells with an antigen against which said T-cell is to be tolerized.

66. The method according to claim 65, wherein activating said dendritic cells comprises administering a glucocorticoid.

67. The method according to claim 66, wherein activating said dendritic cells comprises administering dexamethasone.

68. The method according to claim 65, wherein loading said dendritic cells with an antigen comprises contacting said dendritic cells with cells derived from a graft or transplant donor.

(9) EVIDENCE APPENDIX

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(B) Bruce Alberts et al., Molecular Biology of the Cell (2nd ed. Garland Publishing, Inc., 1989) pp. 1044-1048. First submitted in an IDS mailed November 21, 2003. Resubmitted in an IDS mailed April 27, 2005.

(C) Goodman *et al.*, Goodman and Gillman's The Pharmacological Basis of Therapeutics, (Seventh Ed.) Ch 63 Adrenocorticotrophic Hormone: Adrenocortical Steroids and their Synthetic Analogs: Inhibitors of Adrenocortical Steroid Biosynthesis, page 1464. First presented in the Office Action Response mailed October 29, 2001, and responded to by the Examiner in Office Action mailed September 19, 2002.

(D) Stanford HOPES project. Tan, Glucocorticoids -- Disease Mechanism II: Inflammation (last modified 5-7-03) <http://www.stanford.edu/group/hopes/treatmts/antinflm/i_...>. First submitted in an IDS mailed November 21, 2003, and cited by the Examiner in Office Action mailed October 9, 2007.

(E) Bray *et al.* (1978). Prostaglandin production by macrophages and the effects of anti-inflammatory drugs. Br. J. Pharmacology 63:635-642. First cited by the Examiner in Office Action mailed July 26, 2005.

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Submitted in an IDS mailed November 21, 2003; noted as considered by the Examiner in the Form 1449 initialed December 22, 2004.

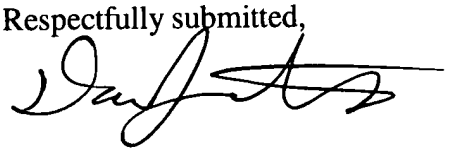
(G) Hancock et al. (1996). Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. Proc. Natl. Acad. Sci. USA. 93:13967-13972. Submitted in an IDS mailed November 21, 2003; noted as considered by the Examiner in the Form 1449 initialed December 22, 2004.

(H) Kampgen *et al.* (1991), Class II Major Histocompatibility Complex Molecules of Murine Dendritic Cells: Synthesis, Sialylation of Invariant Chain, and Antigen Processing Capacity are Down-Regulated Upon Culture. *Proc. Natl. Acad. Sci. USA* 88(8):3014-3018. Submitted in an IDS mailed November 21, 2003; noted as considered by the Examiner in the Form 1449 initialed December 22, 2004.

(10) RELATED PROCEEDINGS APPENDIX

NONE.

Respectfully submitted,



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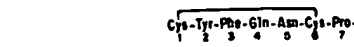
Lypressin

Vasopressin, 8-L-lysine-, Diapid (*Sandoz*)



[50-57-7] $C_{46}H_{65}N_{13}O_{12}S_2$ (1056.22).

Preparation—Isolated from hog pituitaries and prepared synthetically (*J Biol Chem 222:* 951, 1956; *J Am Chem Soc 82:* 3195, 1960). One commercial synthetic method concludes by reacting the protected tripeptide, *N*-tosyl-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanylhydrazide, with the protected hexapeptide, L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl- *N*-tosyl-L-lysyl-glycinamide, and then splits off the protecting groups with metallic sodium.

One mg of lypressin is stated to be equivalent to 270 USP Posterior Pituitary Units (1 Unit is equivalent to 3.7 μg).

Uses—Lypressin has strong antidiuretic but weak pressor activity. It is used only in the treatment of mild to moderate *diabetes insipidus* resulting from neurohypophyseal insufficiency. When the condition is severe, it does not give sufficient control because of its brief duration of action; even in moderate diabetes insipidus, control is only periodic, in accordance with the dosage regimen. In the severe condition, vasopressin tannate is used, although lypressin may be used as an adjunct between injections of the tannate. Diabetes insipidus from renal disorders is not affected. After nasal application of lypressin, antidiuresis peaks within ½ to 2 hr and lasts 3 to 8 hr.

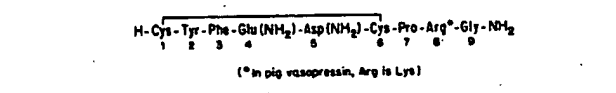
When lypressin is used as recommended, untoward effects are infrequent and mild; they include nasal irritation and congestion, rhinorrhea, nasal pruritus, nasal ulceration, conjunctivitis, and headache. Overdosage may cause heartburn from postnasal drip, abdominal cramps, bowel hypermotility, and fluid retention. Inhalation of the spray can result in asthma-like tightness in the chest, dyspnea, and coughing. Thus far, allergic responses have not been reported. Because the marketed substance is synthetic and thus free of traces of foreign proteins, it may possibly lack allergenicity, even though its composition differs slightly from the human vasopressin.

Dose—*Intranasal*, 1 or 2 sprays (about 7 to 14 μg) to one or both nostrils whenever urinary frequency or substantial thirst indicates a need. The average interval is 6 hours. Increases in dosage should be achieved by more frequent administration rather than by increased number of sprays per application.

Dosage Form—Nasal Spray: 185 μg/mL in 8 mL.

Vasopressin Injection

Beta-Hypophamine; Pitressin (*Parke-Davis*)



8-L-Lysine (or arginine) vasopressin: Lysine form-[50-57-7] $C_{46}H_{65}N_{13}O_{12}S_2$ (1056.22); Arginine form-[113-79-1] $C_{46}H_{65}N_{15}O_{12}S_2$ (1084.23).

A sterile solution in water for injection of the water-soluble, pressor hormone prepared by synthesis or obtained from the posterior lobe of the pituitary of healthy domestic animals used for food by man. Each mL possesses pressor activity equivalent to 20 USP Posterior Pituitary Units and not more than one unit of oxytocic activity.

Description—Clear, colorless or practically colorless liquid with a faint, characteristic odor.

Uses—The actions of vasopressin are discussed on page 957. The injection is employed for its *antidiuretic* effect and to dispel gas shadows in bowel roentgenography and pyelography. It should not be used as a pressor agent.

Untoward effects related to overdosage include water intoxication (with headache, nausea and vomiting, confusion, lethargy, coma, and convulsions), especially when patients drink excessive amounts of water or are given intravenous fluids, and stimulation of vascular, uterine, and intestinal smooth muscle, which may result in pallor, hypertension, coronary constriction (with anginal chest pain, electrocardiographic changes, and occasional myocardial infarction), uterine cramps, menorrhagia, and nausea, vomiting, diarrhea, and abdominal cramps. Hypersensitivity occasionally occurs; manifestations include urticaria, neurodermatitis, flushing, fever, wheezing, dyspnea, and rare anaphylactic shock. Large doses are oxytocic and also cause milk ejection. Alcohol, heparin, demeclocycline, lithium, and large doses of epinephrine antagonize vasopressin; glucocorticoids, urea, and oral hypoglycemic drugs potentiate it.

The plasma half-life is 10 to 20 min. However, the effect of an intramuscular injection lasts from 2 to 8 hr. From 10 to 15% is excreted unchanged.

Dose—*Intramuscular*, for *diabetes insipidus*, 5 to 10 USP Units (0.25 to 0.5 mL) 2 to 8 times a day; for *children*, 2.5 to 10 Units 3 or 4 times a day. May also be given *intranasally* on cotton pledgets, with a dropper, or as a spray, at intervals determined by return of polyuria or thirst. For *hypotonic bowel*, 5 Units, at 3- to 4-hr intervals, as needed.

Dosage Form—20 Units/0.5 and 1 mL.

Other Posterior Pituitary Preparations

Desmopressin Acetate [1-(3-Mercaptopropionic acid)-8-D-arginine vasopressin monoacetate (salt), trihydrate [62357-86-2] $C_{48}H_{68}N_{14}O_{14}S_2.3H_2O$ (1183.22)]—A synthetic analogue of 8-arginine vasopressin (*Helv Chim Acta 49:* 695, 1966) that has the antidiuretic activity of vasopressin but less activity on smooth muscle. It is used in the treatment of *central* ("neurogenic") *diabetes insipidus*. It is also used to test the ability of the kidney to concentrate urine. Since desmopressin can raise the plasma levels of factor VIII (antihemophilic factor), it is sometimes used to increase factor VIII levels prior to surgery. Headache, mild hypertension, nasal congestion, mild abdominal cramping, water intoxication, and vulval pain sometimes occur. Chlorpropamide and clofibrate potentiate, and glyburide inhibits, antidiuretic action. *Dose:* For *diabetes insipidus, intranasal, adult*, 10 to 40 μg a day in 1 to 3 divided doses; *children*, 5 to 30 μg a day. For *urine concentration test, intranasal*, 40 μg. *Dosage Form:* Sterile solution for intranasal use, 0.1 mg desmopressin acetate/mL, 2.5 mL/vial.

Posterior Pituitary [Pituitary; Hypophysis Sicca]—A powder prepared from the clean, dried, posterior lobe of the pituitary of domestic animals used for food by man. Each mg possesses oxytocic activity equivalent to not less than 1 USP Posterior Pituitary Unit. A yellowish or grayish, amorphous powder having a characteristic odor; partially soluble in water. *Uses:* To control postoperative ileus, in surgery as a hemostatic, for diabetes insipidus, and as an oxytocic. The preparation is very little used today. Adverse effects include pallor, hypertension, angina, uterine cramps, anxiety, tinnitus, coma, mydriasis, amaurosis, diarrhea, proteinuria, anaphylaxis, angioneurotic edema, and pruritis. *Dose: Intramuscular* or *subcutaneous*, 5 to 20 Units.

Vasopressin Tannate [β-Hypophamine Tannate; Pitressin Tannate (*Parke-Davis*)]—The water-insoluble tannate of the pressor principle of the posterior lobe of the pituitary of healthy domesticated animals used as food by man. A dark-brown, amorphous solid; insoluble in water and alcohol. *Uses:* For replacement therapy in *diabetes insipidus* but not for its action on the bowel (page 957). Vasopressin tannate has a longer duration of action than vasopressin, and it is more suitable for suspension in oil, so that depot preparations can be made conveniently from the tannate. Because of embolism, vasopressin tannate and its oil suspension should *never be given intravenously*. The untoward effects are the same as those of *Vasopressin Injection*, but the vascular effects are less frequent because of the slower rate of release into the blood stream. *Dose: Intramuscular*, 2.5 to 5 Units (except 1.5 to 2.5 Units in children) every 1 to 3 days. *Dosage Form:* Injection (in oil): 5 Units/1 mL.

The Adrenal Hormones

The adrenal hormones include both the adrenocorticoids from the adrenal cortex and epinephrine and norepinephrine from the adrenal medulla. The discussion below will deal only with the adrenocorticoids. Epinephrine and norepinephrine are treated in Chapter 45.

The cortex, or outer portion of the adrenal gland is one of the endocrine structures most vitally necessary for normal metabolic function. While it is possible for life to continue in the complete absence of adrenal cortical function, serious metabolic derangements ensue, and the capacity of the organism to respond to physiological or environmental stress is completely lost. The vital role of the adrenal cortex is due to its production of a group of hormones, all *steroid* in nature.

Physiology—Four general patterns of adrenal cortical hormone action have been described: (1) retention of sodium ions in extracellular fluid and potassium ions within cells, thus maintaining the normal distribution of water and chloride ion and resulting maintenance of blood volume and blood pressure; (2) maintenance of normal blood glucose levels and facilitation of liver glycogen deposition; and (3) enhanced mobilization of tissue protein and gluconeogenesis from protein; and (4) androgenic effects (see page 997) from androgenic steroids, mainly dehydroepiandrosterone, produced in the adrenal cortex. Steroids that affect the electrolyte metabolism as in (1) are called *mineralocorticoids;* those that affect carbohydrate and protein metabolism as in (2) and (3) and which favor lipolysis are called *glucocorticoids.* Glucocorticoids exert a regulatory influence upon lymphocytes, erythrocytes, and eosinophils of the blood, and upon the structure and function of lymphoid tissue. The relative or complete absence of adrenocortical function, known as *Addison's disease*, is accompanied by loss of sodium chloride and water, retention of potassium, lowering of blood glucose and liver glycogen levels, increased sensitivity to insulin, nitrogen retention, and lymphocytosis. The disturbances in electrolyte metabolism are the cause of morbidity and mortality in most cases of severe adrenal insufficiency. All of these disorders may be corrected by administration of adrenal cortical extract or the pure adrenal cortical steroids now available.

In its biosynthesis of the steroid hormones, the adrenal cortex uses cholesterol, which is present in large amounts in the gland; during periods of secretory activity it also consumes large quantities of ascorbic acid, which is likewise present in high concentration. Control of adrenal gland secretion appears to be of two kinds. The production of mineralocorticoids, chiefly aldosterone, appears to be controlled in part by sodium intake and consequent changes in intravascular fluid volume, in part by the anterior pituitary, and in part by the brain through its modulation of plasma renin activity. For normal development and normal capacity to meet the routine homeostatic requirements, adrenal cortical function must be stimulated by adrenocorticotropin (see *Pituitary Hormones*, page 952); adrenal cortical activity is enhanced through release of corticotropin from the anterior pituitary. In emergency states or during stress, adrenal cortical activity is increased, which prepares for a prolonged duration of the state of stress.

Structures—Over 50 steroids have been shown to be present in the adrenal cortex. Only seven of these, however, have been shown to exert a significant biological effect related to adrenal cortical function. However, the adrenal cortex also produces androgenic steroids. All of the adrenal cortical steroids, except the androgens, contain 21 carbon atoms, an α,β-unsaturated ketone in ring A, and an α-ketol chain ($-COCH_2OH$) attached to ring D. They differ in extent of oxygenation or hydroxylation at carbons 11, 17, or 19. Depending on whether the predominant biological effect is related to electrolyte and water metabolism, or to carbohydrate and protein metabolism, the cortical steroids are classified as either *mineralocorticoid* or *glucocorticoid*, respectively.

In general, clinical experience has indicated that the anti-inflammatory activity of adrenal cortical steroids in man correlates well with their glucocorticoid activity. The undesirable side effects of sodium retention and edema are associated with mineralocorticoid activity. Synthetic steroids possessing higher glucocorticoid and lower mineralocorticoid activity than cortisone or cortisol have been prepared and marketed. All adrenal corticoids require the 3-keto group and 4–5 unsaturation. Additional unsaturation in ring A enhances the anti-inflammatory and antirheumatic properties while at the same time reducing the sodium-retaining effect. Thus, prednisolone has 4 times the anti-inflammatory activity of cortisol and yet has only 0.8 of the mineralocorticoid activity. The presence of oxygen at position 11 is necessary for significant glucocorticoid activity but not for mineralocorticoid activity; the 11β-hydroxy group is more potent than the 11-keto group; the 11-keto group is converted to the active β-hydroxy group in the body. The 17α-hydroxy group is also important to glucocorticoid activity. The 21-hydroxy group is essential to mineralocorticoid activity; it favors but is not required for glucocorticoid activity. Introduction of either methyl or hydroxyl groups at position 16 markedly reduces mineralocorticoid activity but only slightly decreases glucocorticoid and anti-inflammatory activity. Thus, paramethasone (16α-methyl), betamethasone (16β-methyl), dexamethasone (16α-methyl), and triamcinolone (16α-hydroxy) have no significant mineralocorticoid activity. 6α-Methylation has unpredictable effects. It enhances the mineralocorticoid activity of cortisol but virtually abolishes that of prednisolone. The 9α-fluoro group enhances both glucocorticoid and mineralocorticoid activities, but the effects of substituents at the 6 and 16 positions override this effect. Further examples will become apparent from the discussions provided in the following individual monographs.

Biological Activity—For biological testing of adrenal cortical hormones, a number of different types of assays have been used. All of the above compounds will prolong the lives of adrenalectomized animals, although their relative effectiveness varies widely, the mineralocorticoids being 10–30 times as potent as the glucocorticoids. Desoxycorticosterone and aldosterone are likewise considerably more effective in maintaining normal kidney function in adrenalectomized animals. One method of assaying effects on electrolyte metabolism involves the determination of the relative amounts of radioactive sodium (^{24}Na) and potassium (^{42}K) excreted during one hour after their injection. In adrenalectomized animals the ratio (^{24}Na:^{42}K) is high, but is restored to normal upon the injection of microgram amounts of mineralocorticoids. Glucocorticoid activity is most conveniently measured by determining the capacity to restore normal liver glycogen levels in adrenalectomized mice or rats. 17-Hydroxycorticosterone (hydrocortisone or cortisol) and 11-dehydro-17-hydroxycorticosterone (cortisone) are 2 to 5 times as active as corticosterone and 11-dehydrocorticosterone in this test; the 11-desoxycorticoids are essentially inactive. Topical glucocorticoids may also be assayed by their vasoconstrictor effect in the skin of the human.

The glucocorticoids appear to affect all cells, although not all in the same way. Interest primarily focuses on their anti-inflammatory and immunosuppressant effects. They prevent release of various lytic enzymes that not only extend tissue damage during inflammation but also that generate leukotactic substances. They decrease phagocytosis by macrophages and also the disruption of macrophages by ingested materials. Anti-inflammatory effects include the retardation of the migration of polymorphonuclear leukocytes, suppression of repair and granulation, reduction in the erythrocyte sedimentation rate, decreased fibrinogenesis, and diminished elaboration of C-reactive protein. Glucocorticoids do not affect antigen-antibody interaction or the release of the mediators of immediate hypersensitivity. The immunosuppressant effects may be partly the result of the suppression of phagocytosis and immunoinformation processing and partly of a decrease in the number of eosinophils and lymphocytes, suppression of delayed hypersensitivity reactions, decrease in tissue reaction to antigen-antibody interactions (but not in the interaction itself), and a reduction in plasma immunoglobulins.

In addition to the above-mentioned changes brought about by glucocorticoids are the so-called *permissive* effects. In these, the steroids do not themselves cause change but physiological amounts are required for certain organs or structures to respond to stimuli. For example, neither the kidney can

respond to a water load nor the arterioles to epinephrine in the absence of adequate levels of glucocorticoids.

Once a steroid hormone has permeated a cell membrane, it combines with a cytosolic protein called a receptor, or aporeceptor. The steroid-protein complex then translocates to the cell nucleus, where it attaches to chromatin. The result is an enhancement or reduction of the transcription of both messenger RNA and ribosomal RNA, which, in turn, leads to an increased or decreased synthesis of certain proteins. The protein produced is probably determined by the aporeceptor, of which there is more than one kind within the cell. In renal tubular cells, mineralocorticoids appear mainly to induce the synthesis of a protein that decreases intracellular sodium content; it is not clear whether this protein acts directly to increase the activity of membrane [Na^+-K^+]-activated ATPase or indirectly to increase the mitochondrial production of ATP for the ATPase or whether it decreases sodium permease activity. Because of the manifold actions of the glucocorticoids, it is to be expected that many intracellular proteins would be induced, but this has been difficult to verify. Inhibitors of protein- and RNA-synthesis prevent the effects of glucocorticoids, and the cellular content of some enzymes is affected, but with a number of membrane-bound enzymes, only the activities, and not the content, are affected. The activities of some enzymes are increased and others decreased. These are enzymes that have phospholipid adjuvants. Since the glucocorticoids alter the phospholipid composition of a number of cell membranes, it is likely that the enzyme activities are altered through the phospholipid composition, possibly through the induction of phospholipid-synthesizing and/or lytic enzymes. Alterations in membrane composition may possibly explain the membrane-stabilizing effects. Some, but not all, lysosomal membranes are stabilized. Glucocorticoids also inhibit membrane lipid peroxidation, which possibly contributes to the salutary effects in brain edema; the effect appears to be one of decreasing the activity of membrane-bound, superoxide radical-generating mixed-function enzymes. Possibly related is an action to block phospholipase-A_2, which prevents the release of arachidonic acid from membrane phospholipids and its subsequent conversion to hydroxyeicosotetraenoic acid and ultimately prostaglandins, thromboxanes and leukotrienes. Alterations in the prostaglandin system change the cyclic nucleotide balance, often in favor of cyclic guanosyl phosphate. In the proliferative skin diseases, glucocorticoids decrease the markedly elevated arachidonic acid and cyclic AMP levels. Effects on the prostaglandin and cyclic nucleotide systems are undoubtedly involved in the anti-inflammatory response, as is also membrane stabilization. They also induce the production of an antileukokinetic (antichemotactic) peptide. This peptide may prevent the proper assembly of the microtubules, an effect that would also explain the antimitotic effect seen with lymphoblasts and certain other proliferating cells and hence explain certain immunosuppressant and antineoplastic activity and the effect to retard growth. The sundry anabolic (eg, gluconeogenic) and catabolic (eg, proteolytic) effects of the glucocorticoids are also probably indirect.

Side Effects—Certain side effects may appear during the first week of treatment with glucocorticoids; they include euphoria and a rare paradoxical suicidal depression, psychoses (especially with high doses), rare hypertension, anorexia, occasional hyperglycemia, rare colonic ulceration (even though the drugs are used to treat ulcerative colitis), increased susceptibility to infections (especially vaccinial, herpetic, varicellar, and other viral infections, fungal infections, tuberculosis), and acne. They also mask some of the signs of infections, thus causing a postponement of appropriate anti-infective treatment. Glucocorticoids appear to increase peptic ulceration, especially of the stomach. After 2 to 3 days of treatment, the pituitary release of ACTH is suppressed, and the adrenal secretion of cortisol is inadequate once glucocorticoid administration ceases; this condition is temporary after short-term treatment. In the case of a medical emergency, the depressed pituitary-adrenal response may make the patient unable to respond to stress. Consequently, patients on high-dose or long-term treatment should carry a card stating that he/she is under treatment with corticosteroids. Withdrawal of corticosteroids should be slow.

From the first week through the first year of therapy, additional side effects may appear, namely, fat redistribution to the nape of the neck ("buffalo hump") and lower abdomen, diabetes mellitus and hyperglycemia, "moon face" and other edematous states and renal potassium loss (from mineralocorticoid activity), alkalosis, additional infections (including tuberculosis), papilledema, glaucoma, posterior subcapsular cataracts, diplopia, 6th nerve palsies, osteoporosis, myopathy, ecchymoses and purpura, and cutaneous striae. Because of the long-loop negative feedback suppression of ACTH output, the normal adrenal production of corticosteroids from cholesterol is considerably decreased, and hypercholesterolemia results; some of the excess cholesterol is diverted to increase the production of adrenal and testicular androgens, so that masculinization of the female or virilization of the young male and premature cessation of growth may occur. After prolonged suppression of the anterior pituitary secretion of ACTH, there may be a permanent defect in pituitary-adrenal function. Continuous or repetitive use of glucocorticoids may cause painless joint destruction, especially if the drug is given intra-articularly.

After more than a year of glucocorticoid therapy, additional untoward effects include bone fractures and vertebral collapse (from marked osteoporosis), hyperlipidemia, possible premature atherosclerosis, and excessive dependence on the physician. Patients also become physically and psychically dependent upon glucocorticoids and engage in drug-seeking behavior characteristic of addicts.

Adverse effects of glucocorticoids applied to the skin include stinging or burning sensations, itching, irritation, dryness, scaliness, vasoconstriction, folliculitis, acne, bacterial or yeast infections, hypopigmentation, atrophy, and striae. Systemic effects can also occur, especially if occlusive dressings are used. Topical ophthalmologic glucocorticoids not only may cause serious exacerbations of viral, fungal, and bacterial infections of the eye but also glaucoma; examinations for intraocular tension and corneal integrity should be made every 4 to 6 weeks. From all of the above, it can be seen that glucocorticoids are dangerous drugs.

Because the mineralocorticoids are mainly used in physiological doses for replacement therapy, untoward effects are usually infrequent and mild. Sodium and water retention (with "moon face"), potassium loss, alkalosis, and hypertension can occur with excessive doses.

Drug Interactions—Glucocorticoids decrease the hypoglycemic activity of insulin and oral hypoglycemics, so that a change in dose of the antidiabetic drugs may be necessitated. In high doses, glucocorticoids also decrease the response to somatotropin. The usual doses of mineralocorticoids and large doses of some glucocorticoids cause hypokalemia and may exaggerate the hypokalemic effects of thiazide and high-ceiling diuretics. In combination with amphotericin B they also may cause hypokalemia. Glucocorticoids appear to enhance the ulcerogenic effects of nonsteroidal anti-inflammatory drugs. They decrease the plasma levels of salicylates, and salicylism may occur on discontinuing steroids. Glucocorticoids may increase or decrease the effects of prothrombopenic anticoagulants. Estrogens, phenobarbital, phenytoin, and rifampin increase the metabolic clearance of adrenal steroids and hence necessitate dose adjustments.

Other Precautions and Contraindications—Both glu-

cocorticoids and mineralocorticoids must be used cautiously in congestive heart failure, hypertension, liver failure, renal failure, or nephrolithiasis. When glucocorticoids are used in persons with emotional instability or psychotic tendencies, hyperlipidemia, diabetes mellitus, hypothyroidism, myasthenia gravis, osteoporosis, peptic ulcer, ulcerative colitis, chronic infections (especially tuberculosis or a positive test), or a history of herpetic infections, periodic checks should be made. Topical application to the eye is absolutely contraindicated in the presence of ophthalmologic infections.

Absorption and Elimination—All corticosteroids are rapidly and completely absorbed from the gastrointestinal tract. Some, however, particularly the natural ones, are so rapidly destroyed as they pass through the liver that they are poorly effective by the oral route, hence must be given parenterally for systemic effects. Esterification with large hydrophobic organic acids decreases solubility and therefore slows systemic absorption from sites of injection. Esterification with water-soluble acids, such as phosphoric or succinic, increases the rate of absorption from injection sites and may even permit intravenous administration. All of the glucocorticoids are absorbed from the skin, but some slowly enough that metabolic destruction can limit systemic accumulation. Many glucocorticoids are also metabolized in the skin. Fluorination at the 9-position and various substituents at the 17-position make glucocorticoids resistant to local destruction and hence make these derivatives more likely to cause systemic effects. For this reason, topical use is usually avoided in children. In the liver, the carbonyl groups at positions 3, 11, 17, and 20 are reduced to hydroxyl, and the resulting compounds may be conjugated with sulfate or glucuronic acid. Double bonds in the A ring are also reduced. Less than 1% of unchanged steroid is excreted.

In the plasma, corticosteroids are bound to both corticosteroid binding globulin (CBG, transcortin, α_1-globulin) and albumin, which serve as transport vehicles. The extent of binding varies among the steroids. Various drugs and diseases can affect the concentration of transport proteins and their capacities.

Corticoids cross the placental barrier and may cause congenital malformations. They also appear in breast milk and may suppress growth of the infant.

Therapeutic Uses—The adrenal corticosteroids are used for replacement therapy in *adrenal insufficiency* (eg, *Addison's disease* and *congenital adrenal hyperplasia*). In this use, toxic effects are infrequent, since the aim is to approximate the equivalent of physiological body concentrations. Both mineralocorticoids and glucocorticoids may be required; sometimes adrenocortical extracts, which contain both, are used. Glucocorticoids are additionally used to treat rheumatic, inflammatory, allergic, neoplastic, and other disorders; the effects are only palliative and do not eradicate the underlying disorders. It is necessary to use supraphysiological doses, so that some untoward effects are unavoidable.

The anti-inflammatory actions of the glucocorticoids are employed in the treatment of *noninfectious acute ocular inflammation* (*allergic blepharitis*, *iritis*, *uveitis*, *choroiditis*, *conjunctivitis*, *sympathetic ophthalmia*) and certain infectious inflammations, especially in combination with antibiotics. Glucocorticoids are of value, in decreasing some *cerebral edemas*, eg, vasogenic, but are of dubious value in cerebral edema from other causes. In *infantile massive spasms* (minor motor epilepsy) glucocorticoids may be of benefit, but it is not clear how this derives from anti-inflammatory activity. In serious *acute allergic disorders*, glucocorticoids may be indicated; they should not be used chronically in allergic disorders, except in acute flare-ups. (However, they are approved for intranasal application for chronic noninfectious rhinitis.) Similarly, *acute bronchial asthma*, *status asthmaticus*, and some chronic *obstructive pulmonary disease* may require glucocorticoids, but they should be avoided, if possible, in chronic asthma because of the implications for lifetime medication. These drugs suppress allergic and inflammatory manifestations of *trichinosis*.

Topical or systemic glucocorticoids often markedly improve certain skin diseases, such as *pruritus*, *psoriasis*, *dermatitis herpetiformis*, and *eczema*; *pemphigus*, *erythema multiforme*, *exfoliative dermatitis*, and *mycosis fungoides* usually require systemic treatment, which may be life-saving.

Probably the most widely known application of the anti-inflammatory actions of the glucocorticoids is in the treatment of the arthritides and rheumatic disorders. Immunosuppressant actions may also play a role in the treatment of such disorders. These disorders are *systemic lupus erythematosus*, *polyarteritis*, *temporal arteritis*, *Wegener's granulomatosis*, *polymyositis*, and *polymyalgia rheumatica*. Glucocorticoids may be indicated in severe cases of *rheumatoid arthritis* unresponsive to other treatment, *Still's disease*, *mixed connective tissue disease*, *drug-induced lupoid syndromes*, and *psoriatic arthropathy*. Rheumatic or arthritic conditions in which glucocorticoids may or may not provide temporary relief but are not justified chronically because of a high toxicity:benefit ratio are osteoarthritis, systemic ankylosing spondylitis, gout fibrositis, and Reiter's syndrome. Even though the *nephrotic syndrome* is not inflammatory, it may respond to treatment, perhaps as the result of immunosuppression. *Ulcerative colitis* sometimes may respond dramatically. The beneficial effects in *myasthenia gravis* are probably immunosuppressant. Chronic multiple sclerosis does not respond but acute relapses may.

Glucocorticoids may be palliative in *acute leukemia* and also in *chronic lymphocytic leukemia*, and they are components of certain curative antineoplastic combinations. They suppress the associated autoimmune hemolytic anemia and the nonhemolytic anemia, granulocytopenia, and thrombocytopenia that result from encroachment on the bone marrow, and also the cachexia and fever. The effects are only temporary, and the patient eventually becomes refractory to steroid therapy. *Hodgkin's disease*, *lymphosarcoma*, and *multiple myeloma* may also be temporarily suppressed, though most frequently only the pain is diminished. The mechanism of the palliative effects on these neoplasms is unknown.

In the treatment of *endotoxin shock*, massive doses of glucocorticoids suppress the vasculotoxic effects of the toxin. In all kinds of *shock*, massive doses decrease peripheral resistance, stimulate the heart, and decrease the amount of circulating myocardial depressant factor. To be optimally effective they must be given as a bolus.

Modalities and Regimens of Corticosteroid Therapy—*Replacement Therapy*—Treatment of primary and secondary adrenal insufficiency requires replacement of both glucocorticoids and mineralocorticoids in sufficient doses to relieve the signs and symptoms of insufficiency. However, when the patient experiences an additional stress, supplements of glucocorticoids may be required. The dose and dose-interval vary from patient to patient, but the doses are small and complications are infrequent and minimal; the most difficult challenge is in the adjustment of dosage in response to changes in stress.

Chronic Low-Dose Systemic Therapy of Disease—In mild inflammatory or collagen disorders, low doses of glucocorticoids are often sufficient to be palliative, and low-dose regimens are preferable, since adverse effects usually are of low intensity, provided that the therapeutic end point is only an amelioration and not elimination of the morbidity. Although low-dose therapy may cause some suppression of pituitary-adrenal function, the suppression is readily reversible, and some reserve in the system is extant. However, abrupt withdrawal of the drug not only may be followed by a return

to the previous condition but an acute exacerbation of the disease. Pituitary-adrenal suppression and consequent acute flare-up after withdrawal may be lessened by avoiding round-the-clock administration and, instead, giving the drug between 8 and 9 am, in order that plasma levels and hence pituitary-adrenal suppression be at a minimum during the early morning sleeping hours, when pituitary adrenal function is at its diurnal peak.

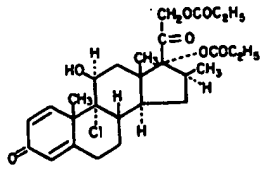
Chronic High-Dose Systemic Therapy—In serious chronic inflammatory or immunologic disorders or in glucocorticoid-responsive neoplasia, large doses of glucocorticoids may be given for long periods of time. Consequently, side effects are frequent, and pituitary-adrenal suppression may be severe. The suppression may continue for weeks to months after cessation of treatment, so that withdrawal must be slowly tapered off to allow the pituitary-adrenal system to recover. Abrupt withdrawal will result in adrenal insufficiency, which may be life-threatening, as well as an acute recrudescence of the original disorder. Pituitary-adrenal suppression and systemic side effects may be less severe if the dose is given in the morning, so that nocturnal pituitary-adrenal activity is less inhibited. Another device to minimize such adverse systemic effects is that of *alternate day therapy*, in which twice the usual daily dose is given but only every other day, which permits the hypothalamico-pituitary segment of the pituitary-adrenal negative feedback system and various undiseased target organs time to recover partly toward normal between doses.

Intensive Short-Term Systemic Therapy—Massive doses of glucocorticoids may be required in certain acute conditions, such as bacteremic shock, status asthmaticus, etc. The short duration of such treatment, sometimes no longer than 48 hr, is not enough to give rise to pituitary-adrenal suppression, serious immunosuppression, or opportunistic infections, although in septic shock, suprainfections may occur. Psychosis, gastrointestinal bleeding, and hyperosmolar diabetic coma can occur in such short-term use.

Local Treatment—TOPICAL APPLICATION. Topical efficacy depends on the inherent glucocorticoid activity (or potency) of the steroid, the concentration in the preparation, permeability coefficient, the vehicle and excipients, and local metabolic processes. Except for serious conditions, low-potency glucocorticoids are preferred by many authorities, because adverse effects on the skin appear to be less severe than with high-potency agents, even if the latter are used at appropriately lower concentrations. Only hydrocortisone and its acetate are available for nonprescription topical use. Drugs with a high lipid-water distribution coefficient penetrate well from absorbable or nonoleaginous vehicles and tend to remain longer in the skin than water-soluble agents, exerting a more extended local action but lesser systemic side effects, especially if the drug is rapidly metabolized systemically. However, it is desirable that the agents be metabolized in the skin, so that less is delivered to the systemic circulation. Steroids that have the 17-OH group substituted and/or which are fluorinated are poorly metabolized locally and hence may have a significant potential for systemic effects; for this reason, especial caution is urged when such compounds are used in children. Occlusive dressings may be used, especially for low-potency, poorly penetrant steroids. The stratum corneum under the dressing becomes macerated and more permeable. However, such dressings increase absorption into the blood stream and hence favor systemic effects. LOCAL INJECTION. In order to achieve high, rapidly acting local concentrations of a glucocorticoid, it is sometimes injected as a very soluble derivative which rapidly generates the parent steroid. However, such soluble forms also rapidly leave the region of injection. For this reason, insoluble derivatives may be included or injected alone, so that a sustained action in parallel with slow dissolution may be effected.

Beclomethason Dipropionat

Pregna-1,4-diene-3,20-dione, 9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11β,16β)-, Vanceril (*Schering-Plough*)



9-Chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,-20-dione 17,21-dipropionate [5534-09-8] $C_{28}H_{37}ClO_7$ (521.05).

Preparation—Synthesis of beclomethasone, a 9-chloro-16β-methyl derivative of prednisolone, and esters of beclomethasone, from steroid intermediates is described in British Pats 901,093 and 912,378 (*CA 58:* 3488e, 1963; *59:* 14082b, 1963).

Description—White to cream-white powder; odorless.

Solubility—Very slightly soluble in water; very soluble in chloroform; freely soluble in alcohol and acetone.

Uses—Beclomethasone dipropionate has 500 times the topical anti-inflammatory activity of dexamethasone but is less active as a systemic glucocorticoid and is almost inactive by the oral route. The low systemic activity is the result of rapid de-esterification and further metabolism in the liver. Also, it has a high lipid- but low water-solubility, so that it not only is well absorbed topically but also tends to remain at the site of application. Thus it may be administered by oral inhalation with usually negligible systemic side effects. It is indicated only in the treatment of *bronchial asthma* in which bronchodilators and cromolyn sodium are ineffective. As long as 2 to 4 weeks may be required for the onset of a beneficial effect. It is also employed in the treatment of *noninfectious rhinitis*.

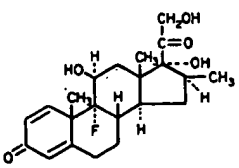
The most common side effects of inhaled beclomethasone are dry mouth, hoarseness, sore throat, and pharyngeal or tracheal candidiasis. Usually, the effects on pituitary-adrenal function are negligible, but suppression of plasma cortisol levels occurs in a few percent of adult patients who receive 1600 μg/day and in all who receive 4000 μg/day. In children, doses of 400 to 800 μg/day have effects on adrenal function comparable to alternate-day therapy with prednisone. Patients who switch from continuous oral glucocorticoids to beclomethasone aerosols often show signs and symptoms of systemic glucocorticoid deficiency, and deaths from adrenal insufficiency have been reported. Adverse effects of intranasal beclomethasone include epistaxis, nasal irritation, sneezing and nasopharyngeal candidiasis. Hypersensitivity or other adverse effects to the propellants (CHF_3 and CH_2F_2) and oleic acid (a dispersing agent) may occur; hypersensitivity absolutely contraindicates use of the aerosol. The effects of beclomethasone on the fetus *in utero* and the extent of secretion into milk are not yet known.

Dose—*Oral inhalation, adults* and *children over 12 yrs* of age, *initially*, 84 μg (2 metered inhalations) 3 or 4 times a day, to be adjusted later to the minimal number of daily inhalations that will control symptoms; in severe asthma, the initial dose is 100 μg 6 to 8 times a day and subsequently adjusted as needed, usually to a lower dose but occasionally to doses as high as 1000 μg per day or more; *children 6 to 12 yrs* of age, 42 to 84 μg (1 to 2 metered sprays) 3 or 4 times a day, not to exceed 500 μg per day. *Nasal insufflation, adults* and *children over 12 yrs* of age, *initially* 42 μg (1 metered insufflation) in each nostril 2 to 4 times a day; *maintenance*, 3 times a day. The mouth and throat should be rinsed after each oral or intranasal insufflation.

Dosage Form—Aerosol: 16.8 g/canister; each metered spray releases approximately 42 μg.

Betamethasone

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16β)-, Celestone (*Schering-Plough*)



9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione [378-44-9] $C_{22}H_{29}FO_5$ (392.47).

Preparation—Betamethasone is prepared from 16-dehydropregnenolone (see *Progesterone*, page 993) by treatment with methyl magnesium iodide to insert the 16β-methyl group, catalytic reduction of the remaining double bond, enol acylation at position 20, and reaction with peracetic acid followed by hydrolysis to the 16β-methyl-17α-hydroxy compound. Bromination and acetoxylation gives the 3β-hydroxy-21-acetoxy derivative which is oxidized to the 3-oxo compound with chromic acid. Dibromination at positions 1 and 4 followed by dehydrobromination with dimethylformamide to the 1,4-diene, then incubation with *Pestalotia foedans* (or a similar organism) results in the 11α-hydroxy derivative. Esterification at the 11-position with ethyl chloroformate, elimination of the ester function with acetic acid to form the 1,4,9(11)-triene, treatment with *N*-bromoacetamide and perchloric acid gives the 9α-bromo-11β-hydroxy compound. Abstraction of HBr with potassium acetate affords the 9β,11β-epoxy derivative which by treatment with HF in a halogenated hydrocarbon yields the 9α-fluoro-11β-hydroxy analogue, betamethasone.

Description—White to practically white, odorless, crystalline powder; melts at about 240° with some decomposition.

Solubility—1 g in 5300 mL water, 65 mL alcohol, 325 mL chloroform; very slightly soluble in ether.

Uses—An extremely potent glucocorticoid with actions, uses, and side effects typical of this class of steroids (see introduction to this section). Its activity is 20 to 30 times that of cortisol. However, it only rarely induces sodium and water retention and potassium loss such as accompany treatment with cortisone and many other adrenal corticoids; on occasion, betamethasone may even increase sodium excretion and induce diuresis. In the usual doses, the incidence of characteristic adrenal corticoid untoward effects such as anorexia, protracted weight loss, vertigo, headache, and muscle weakness is quite low. The serum half-life of betamethasone is about 3 hr.

Dose—*Oral, adult, initially*, 0.6 to 7.2 mg (usually 2.4 to 4.8 mg) daily in single or divided doses; *maintenance*, 0.6 to 1.2 mg daily or on alternate days; *pediatric, replacement*, 17.5 μg per kg of body weight or 500 μg per m^2 of body surface a day in 3 divided doses, and, for *disease*, 62.5 to 250 μg/kg or 1.88 to 7.5 mg/m^2 in 3 or 4 divided doses. *Topical*, as 0.2% cream applied to skin 2 or 3 times a day in adults and once a day in children.

Dosage Forms—Cream: 0.2%; Syrup: 0.6 mg/5 mL; Tablets: 0.6 mg.

Betamethasone Acetate

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(acetyloxy)-, (11β,16β)-, Betamethasone 21-Acetate

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,-20-dione 21-acetate [987-24-6] $C_{24}H_{31}FO_6$ (434.50).

For the structure of the base, see *Betamethasone*.

Preparation—*Betamethasone* 962 is acetylated with acetic anhydride in the presence of pyridine. US Pat 3,164,618.

Description—White to creamy white, odorless powder; sinters and resolidifies at about 165° and remelts with decomposition between 200° and 220°.

Solubility—1 g in 2000 mL water, 9 mL alcohol, 16 mL chloroform.

Uses—The actions are the same as those of the parent compound, *Betamethasone*. However, at present, the acetate is marketed only in combination with the sodium phosphate. The acetate is less soluble than the sodium phosphate, so that the acetate provides a sustained action after intramuscular or intra-articular injection.

Dose—See *Sterile Betamethasone Sodium Phosphate and Betamethasone Acetate Suspension*.

Betamethasone Sodium Phosphate

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11β,16β)-, Betamethasone 21-(Disodium Phosphate)

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,-20-dione 21-(disodium phosphate) [151-73-5] $C_{22}H_{28}FNa_2O_8P$ (516.41).

For the structure of the base, see *Betamethasone*.

Preparation—Starting with *Betamethasone*, by the method described for *Dexamethasone Sodium Phosphate*. US Pat 3,164,618.

Description—White to practically white, odorless powder; hygroscopic.

Solubility—1 g in 2 mL water, 470 mL alcohol, >10,000 mL chloroform, >10,000 mL ether.

Uses—The actions are those of *Betamethasone*, to which the more soluble disodium phosphate is converted in the body. Following injection, the plasma or synovial fluid levels rise at a rapid rate to high levels, which effects a prompt response. Parenteral betamethasone sodium phosphate is employed when oral glucocorticoids cannot be used or when it is desirable to inject the drug directly into the affected structure.

Dose—The following doses are stated in terms of the *betamethasone equivalents*, but the content of ampules and vials is stated for betamethasone sodium phosphate itself. *To convert*, 4 mg of the sodium phosphate derivative is equivalent to 3 mg of betamethasone. *Intramuscular* or *intravenous, adult, initially* up to 9 mg/day, to be adjusted downward as the disease responds; *intra-articular, intralesional*, or into *soft tissue*, up to 9 mg/day as needed. *Children, intramuscular*, for *replacement*, 17.5 μg/kg or 500 μg/m^2 of body weight every third day *or* 5.8 to 8.75 μg/kg or 166 to 250 μg once a day; for *disease*, 20.8 to 125 μg/kg or 625 μg to 3.75 mg/m^2 once or twice a day.

Dosage Forms—Injection: 4 mg/1 mL and 20 mg/5 mL, respectively equivalent to 3 mg/1 mL and 15 mg/5 mL of betamethasone. See also *Sterile Betamethasone Sodium Phosphate and Betamethasone Acetate Suspension*.

Sterile Betamethasone Sodium Phosphate and Betamethasone Acetate Suspension

Celestone Soluspan (*Schering*)

A sterile preparation of betamethasone sodium phosphate in solution and betamethasone acetate in suspension in water for injection.

Uses—See *Betamethasone Acetate* and *Betamethasone Sodium Phosphate*. The combination is intended for use in glucocorticoid-responsive disease (see page 961) in patients in whom oral medication cannot be achieved, in acute self-limiting disease in which a single dose is sufficient, and to initiate treatment in severe diseases where a prompt response is desired prior to switching to a drug with a slower onset and longer duration of action. Although the injection may be given intra-articularly, it must be remembered that repeated intra-articular glucocorticoids sometimes permit painless destruction of the joint. The suspension contains benzalkonium chloride, which may sensitize some recipients.

Dose—Doses are stated in terms of total glucocorticoid content. *Adult, intra-articular*, 1.5 to 12 mg and *intrabursal*, 6 mg, repeated as needed; *intradermal* or *intralesional*, 1.2 mg/cm^2 of affected skin, up to a total of 6 mg, to be repeated once a week, if necessary; *intramuscular*, 500 μg to 9 mg/day. Pediatric doses not determined. Although the manufacturer's recommendations for parenteral therapy are for divided doses at 12-hr intervals, it is advisable to give all or nearly all of the daily dose between 8 and 9 am to minimize interference with the nocturnal activity of the pituitary-adrenal system.

Dosage Form—Sterile Suspension: Betamethasone phosphate equivalent to 15 mg of betamethasone and 15 mg of betamethasone acetate/5 mL.

Betamethasone Valerate

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11β,16β)-, Bethamethasone 17-Valerate; Valisone (*Schering-Plough*)

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,-20-dione 17-valerate [2152-44-5] $C_{27}H_{37}FO_6$ (476.58).

For the structure of the base, see *Betamethasone*.

Preparation—A solution of *Bethamethasone* in an organic solvent is treated with a lower alkyl orthovalerate such as trimethyl orthovalerate [$C_4H_9C(OCH_3)_3$] to produce betamethasone-17,21-ylene alkyl orthovalerate. This is then hydrolyzed with dilute acid and the resulting crude betamethasone 17-valerate is extracted and crystallized from a suitable organic solvent. US Pat 3,312,590.

Description—White to practically white, odorless, crystalline powder; melts at about 190°, with decomposition.

Solubility—1 g in 10,000 mL water, 16 mL alcohol, <10 mL chloroform, 400 mL ether.

Uses—The actions are the same as those of the parent compound, *Betamethasone*. However, the physicochemical properties of the compound favor penetration into the skin. It is thus employed for treatment of inflammatory and allergic dermatoses and dermatitides (see the general statement in this section).

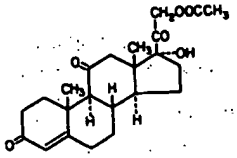
Unless extensive areas of the skin are dressed with betamethasone valerate cream under occlusion, systemic effects are unlikely to occur. However, prolonged topical use may cause cutaneous and subcutaneous atrophy and consequent striae. Irritation, folliculitis, and sensitization are rare.

Dose—*Topical, adult*, as a cream, lotion, or ointment containing the equivalent of 0.01 or 0.1% of betamethasone to the affected area 1 to 3 times a day or as an aerosol containing the equivalent of 0.15% betamethasone 3 to 4 times a day. *Children*, as a 0.01% cream 1 or 2 times a day or as a 1% cream, lotion, or ointment, or 0.15% aerosol once a day.

Dosage Forms (as the equivalent of betamethasone)—Aerosol: 0.15%; Cream: 0.01 and 0.1%; Lotion: 0.1%; Ointment: 0.1%.

Cortisone Acetate

Pregn-4-ene-3,11,20-trione, 21-(acetyloxy)-17-hydroxy-, Kendall's Compound E Acetate; Wintersteiner's Compound F Acetate; Reichstein's Substance Fa Acetate; Cortone Acetate (*MSD*)



17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate [50-04-4] $C_{23}H_{30}O_6$ (402.49).

Preparation—By a variety of methods using easily obtainable starting materials such as ergosterol, diosgenin or hecogenin from plant materials and cholesterol or desoxycholic acid from animal sources. The cortisone is esterified with acetic anhydride to give the acetate.

Description—White or practically white, odorless, crystalline powder; stable in air; melts at about 240° with some decomposition.

Solubility—Insoluble in water; 1 g in about 350 mL alcohol; 4 mL chloroform, 30 mL dioxane, and 75 mL acetone.

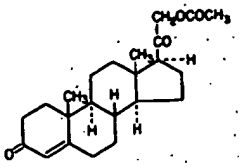
Uses—Cortisone is a natural glucocorticoid with a slight degree of mineralocorticoid activity; it has 0.8 the glucocorticoid activity of cortisol. Cortisone acetate is used specifically, in combination with desoxycorticosterone acetate, in *adrenal cortical insufficiency*. It may also be used for the numerous purposes described in the introduction to this section, where its untoward effects are also described. Although cortisone acetate is applied locally in some conditions, it requires conversion to cortisol to be substantially effective, and there is little to justify topical use. The plasma half-life of cortisone is about 30 min, which is shorter than the half-time for absorption of the acetate and conversion to cortisone. The plasma half-time for hydrocortisone, the active form of cortisone, is 1½ to 2 hr.

Dose—*Oral, adult*, for *replacement therapy*, 20 to 70 mg daily, ⅔ taken in the morning and ⅓ in the afternoon, and, for *anti-inflammatory effects*, 25 to 50 mg daily in mild chronic disorders and 75 to 300 mg in acute and severe chronic disorders. *Children, replacement*, 700 μg/kg of body weight (or 20 mg per m^2 of body surface) a day every third day or 233 to 350 μg/kg or 6.66 to 10 mg/m^2 of body surface once a day; for *anti-inflammatory effects*, 2.5 to 10 mg/kg of body weight (or 75 to 300 mg/m^2 of body surface) a day. *Intramuscular, adult*, 20 to 30 mg a day for serious chronic disorders; absorption is too slow and erratic by this route to lend itself to treatment of acute disorders. Once a satisfactory initial response has been obtained, the dose is gradually lowered to the satisfactory maintenance minimum. *Topical*, to the *conjunctiva, adult* and *children*, as a 1.5% ointment, 3 or 4 times a day.

Dosage Forms—Ophthalmic Ointment: 1.5%; Sterile Suspension: 250 mg/10 mL and 500 mg/10 mL; Tablets: 5, 10, and 25 mg.

Desoxycorticosterone Acetate

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-, Desoxycortone Acetate; Deoxycortone Acetate; Doca Acetate (*Organon*); Percorten (*Ciba*)



11-Deoxycorticosterone acetate [56-47-3] $C_{23}H_{32}O_4$ (372.50).

Preparation—Desoxycorticosterone, synthesized from the soya bean phytosterol stigmasterol, is condensed with acetyl chloride in pyridine solution. Dilution of the reaction product with water precipitates the ester.

Description—White, or creamy white, crystalline powder; odorless and stable in air; melts between 155° and 161°.

Solubility—Practically insoluble in water; sparingly soluble in alcohol, acetone, and dioxane; slightly soluble in vegetable oils.

Uses—A natural mineralocorticoid (see page 958 for actions, uses, and side effects). Physiologically it is of much less importance than aldosterone, but the cost of the latter is too prohibitive for clinical use. Treatment of *Addison's disease* has been greatly advanced by the use of desoxycorticosterone. Although the defects in carbohydrate and protein metabolism are not corrected by this particular compound, life can be maintained by its intelligent administration.

The serum half-life of desoxycorticosterone is about 70 min. Since Addison's disease is a permanent disorder, treatment is for life, and a long duration of action is therefore desirable. Desoxycorticosterone sometimes is administered in the form of subcutaneous pellets. With pellet implantation the hormone is slowly absorbed, and a single implantation of an adequate number of pellets may be effective for as long as 6 months or more. Signs of underdosage are corrected with intramuscular administration, and signs of overdosage by salt restriction. Addisonian patients usually take cortisone acetate or hydrocortisone along with desoxycorticosterone.

Patients receiving desoxycorticosterone should be careful to maintain an adequate intake of salt and carbohydrate and, during periods of acute adrenal insufficiency, carbohydrate must be specially administered. In crisis, extracts containing glucocorticoids should be administered.

Dose—*Intramuscular, adults*, for *Addison's disease*, initially 2 to 10 mg/day, then 1 to 5 mg/day for maintenance; for salt-losing *adrenogenital syndrome*, up to 6 mg a day for 3 or 4 days, after which dosage is adjusted according to clinical response; *children*, 1 to 5 mg/day or 1.5 to 2 mg/m^2/day, with frequent monitoring. *Subcutaneous implantation, adults* 1 pellet (125 mg) for each 0.5 mg of the injection required for daily maintenance, repeated at 8- to 12-month intervals.

Dosage Forms—Injection: 50 mg/10 mL; Pellets: 125 mg. The injection contains parabens, which can cause sensitization.

Desoxycorticosterone Pivalate

Pregn-4-ene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-, Percorten Pivalate (*Ciba*)

11-Deoxycorticosterone pivalate [808-48-0] $C_{28}H_{38}O_4$ (414.59).

For the structure of the base, see *Desoxycorticosterone Acetate*.

Preparation—As described for the acetate except that trimethylacetyl chloride is employed.

Description—White, or creamy white, crystalline powder; odorless and stable in air; melts between 200° and 206°.

Solubility—Practically insoluble in water; soluble 1 g in 450 mL alcohol, 3 mL of chloroform, 160 mL of methanol. Soluble in ether and fixed oils.

Uses—The actions are the same as those of *Desoxycorticosterone Acetate*, except that intramuscular microcrystalline suspensions of the trimethylacetate (pivalate) have a very long duration of action. Consequently, it is used only for the treatment of chronic primary and secondary adrenal cortical insufficiency, usually only after a maintenance dose of desoxycorticosterone acetate is first established.

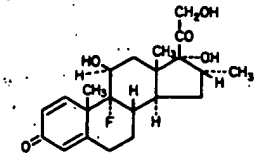
Dose—*Intramuscular, adults* and *children* 25 mg for each mg of the oil solution of desoxycorticosterone acetate required for mainte-

nance in the trial regimen (usually 25 to 100 mg), administered every 4 weeks.

Dosage Form—Sterile Suspension: 100 mg/4 mL.

Dexamethasone

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16α)-, (*Various Mfrs*)



9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,-20-dione [50-02-2] $C_{22}H_{29}FO_5$ (392.47).

Preparation—In a manner quite similar to that for *Betamethasone*, the difference being that the 16-methyl group is inserted in the α-configuration.

Description—White to practically white, odorless, crystalline powder; stable in air; melts at about 250° with some decomposition.

Solubility—Soluble 1 g in 42 mL of alcohol, 165 mL of chloroform; sparingly soluble in acetone, dioxane, and methanol; very slightly soluble in ether; practically insoluble in water.

Uses—Dexamethasone possesses glucocorticoid activity, for which it is used clinically (see introduction to this section). It is especially used as an anti-inflammatory and antiallergic drug. Topically it is employed in the treatment of glucocorticoid-responsive dermatoses. Its systemic glucocorticoid potency is about 25 times that of cortisone. It is capable of inducing all the usual side effects of adrenal corticoids, except that the mineralocorticoid-like side effects are less pronounced than with cortisone acetate.

Its effect to suppress pituitary-adrenocortical function is used for differential diagnostic purposes in Cushing's syndrome. In the *rapid overnight test*, 1 mg of dexamethasone given at 11 or 12 pm will have a marked suppressant effect on plasma cortisol levels at 8 am in persons who do not have Cushing's syndrome but little effect on those who do. In the *low-dose 2-day test*, 0.5 mg every 6 hr for 2 days will fail to suppress 24-hr urinary output of 17-hydroxysteroids in patients with bilateral adrenal hyperplasia and autonomous adenomas but not in others. In the *high-dose 2-day test*, 2 mg every 6 hr for 2 days will suppress urinary 17-hydroxysteroids in adrenal hyperplasia (except multinodular hyperplasia) and most ACTH-responsive adrenal adenomas. Some multinodular hyperplasias and ACTH-responsive adrenal adenomas will not show suppression until the dose of dexamethasone is increased to 4 to 8 mg every 6 hr. When adrenal hyperplasia is secondary to an ACTH-producing tumor, no suppression will occur in any of these tests.

The plasma half-life of dexamethasone is 3 to 4 hr.

Dose—*Oral, adult, initially* 500 μg to 9 mg a day in single or divided doses, and usually less for *maintenance;* for *diagnostic doses*, see *Uses*, above; *children*, for *replacement*, 23 to 330 μg per kg of body weight (or 670 μg to 10 mg per m² of body surface) a day, and, for *disease*, 83 to 333 μg/kg (or 2.5 to 10 mg/m²) a day in 3 or 4 divided doses. *Topical*, to the *skin*, as 0.01% aerosol 2 to 4 times a day, 0.04% cream or 0.1% gel 3 or 4 times a day, except only 1 or 2 times a day with any preparation in children. *Topical*, to the *conjunctiva*, 1 drop of 0.1% suspension 3 or 4 times a day.

Dosage Forms—Aerosol: 0.01%; Cream: 0.04%; Elixir: 0.5 mg/5 mL; Gel: 0.1%; Ophthalmic Suspension: 0.1%; Tablets: 0.25, 0.5, 0.75, 1.5, 4 and 6 mg.

Dexamethasone Sodium Phosphate

Pregn-4-ene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11β,16α)-, Dexamethasone 21-(Disodium Phosphate); Decadron Phosphate (*MSD*); Dalalone (*O'Neal, Jones & Feldman*)

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium salt [2392-39-4] $C_{22}H_{28}FNa_2O_8P$ (516.41).

For the structure of the base, see *Dexamethasone*.

Preparation—Dexamethasone is esterified with methanesulfonyl chloride at the 21-position, and the ester is refluxed with sodium iodide in ethanol to form the 21-iodo derivative. This is treated with silver dihydrogen phosphate and the resulting 21-(dihydrogen phosphate) is neutralized with sodium hydroxide.

Description—A white, or slightly yellow, crystalline powder; odorless or has a slight odor of alcohol; very hygroscopic; pH (1 in 100 solution) between 7.5 and 10.5.

Solubility—1 g dissolves in about 2 mL of water; slightly soluble in ether and chloroform.

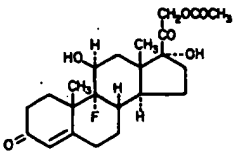
Uses—The same actions as *Dexamethasone*. It is one of the most soluble adrenocortical compounds. Thus it lends itself well to intravenous administration, local injection, inhalation, and to solutions and water-based ointments for topical application, especially for ophthalmologic use. The inhalation aerosol is used in the management of bronchial asthma. Although it may be given intra-articularly, it is usually not recommended by this route because of the danger of painless joint destruction. The adverse effects and contraindications are those of other glucocorticoids (see the introduction in this section).

Dose—All doses below are stated in terms of dexamethasone equivalents. *Intravenous* or *intramuscular, adult*, 420 μg to 7.5 mg per day, the dosage being decreased when a response occurs; *intra-articular, intralesional*, or *soft-tissue injection*, 170 μg to 5 mg. *Oral inhalation*, 3 metered sprays (252 μg) in *adults and* 2 metered sprays (168 μg) in *children*, 3 or 4 times a day. *Intranasal, adult*, 2 metered sprays (168 μg) into each nostril 2 or 3 times a day up to 1.2 mg/day; *children* over 6 years of age, 1 or 2 metered sprays into each nostril twice a day. *Topical*, to the *skin*, as 0.1% cream, 3 or 4 times a day for *adults* or once a day for *children;* to the *conjunctiva* as 0.05% ointment 3 or 4 times a day or 1 drop of 0.05% solution 4 to 6 times a day.

Dosage Forms—(as dexamethasone equivalents) Inhalation Aerosol: 84 μg/metered spray; Nasal Aerosol: 84 μg/metered spray; Cream: 0.1%; Injection: 3.3, 8.33 and 20 mg/mL; Ophthalmic Ointment: 0.05%; Ophthalmic Solution: 0.1%.

Fludrocortisone Acetate

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β)-, Florinef Acetate (*Squibb*)



9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate [514-36-3] $C_{23}H_{31}FO_6$ (422.49).

Preparation—One method starts with *Cortisol Acetate* which is first dehydrated to the 4,9-diene. The 9α-fluoro and 11β-hydroxy groups are inserted by a method similar to that used for *Betamethasone*.

Description—Fine, white to pale-yellow powder that is odorless or practically odorless; hygroscopic; melts at about 225° with some decomposition.

Solubility—Insoluble in water; soluble 1 g in 50 mL of alcohol, 50 mL of chloroform, or 250 mL of ether.

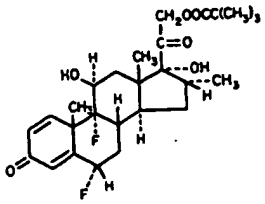
Uses—A potent mineralocorticoid with considerable glucocorticoid activity. Its uses and side effects are those of mineralocorticoids (see page 958), except that when used for replacement therapy in adrenal insufficiency it may not always be necessary to use a glucocorticoid concurrently, although usually cortisol or cortisone are also administered. With the doses used for replacement therapy, glucocorticoid side effects of fludrocortisone acetate alone are mild and infrequent. The plasma half-life is about ½ hour.

Dose—*Oral, adult*, for *chronic adrenal insufficiency*, 0.05 to 0.2 mg once a day to 3 times a week; for *congenital adrenogenital syndromes*, 0.1 to 0.2 mg/day. In both of the above, a glucocorticoid is usually concomitantly administered.

Dosage Form—Tablets: 0.1 mg.

Flumethasone Pivalate

Pregna-1,4-diene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-6,9-difluoro-11,17-dihydroxy-16-methyl-, (6α,11β,16α)-, Locorten (*Ciba-Geigy*); Locacorten (*Ciba-Geigy*)



6α,9-Difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate [2002-29-1] $C_{27}H_{36}F_2O_6$ (494.57).

Preparation—The method described for *Betamethasone* (page 902) may be modified appropriately to cause the 16-methyl group to enter in α-configuration and to include one of several known methods for introducing the 6α-fluorine. The resulting flumethasone may be converted to the 21-pivalate by treatment with trimethylacetic anhydride in the presence of pyridine.

Description—White to off-white, crystalline powder.

Solubility—Practically insoluble in water; soluble 1 g in 90 mL of alcohol, 350 mL of chloroform, or 2800 mL of ether.

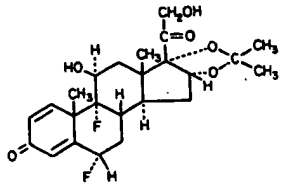
Uses—A glucocorticoid with about 800 times the potency of cortisone acetate. It is used only topically, for the treatment of glucocorticoid-responsive *dermatological disorders* (see the introduction in this section). When applied under occlusive dressings, it is especially used in the treatment of *nummular dermatitis*, *psoriasis* and *chronic neurodermatitis*. Since it contains fluorine substituents, metabolism in the skin is slow; nevertheless, systemic elimination is fast enough that systemic side effects are weak.

Dose—*Topical*, as 0.03% cream applied as a film 3 or 4 times a day or under an occlusive dressing in *adults*, but only once a day in *children*.

Dosage Form—Cream: 0.03%.

Fluocinolone Acetonide

Pregna-1,4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-,
Fluonid (*Herbert*); Synalar (*Syntex*)



6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone [67-73-2] $C_{24}H_{30}F_2O_6$ (452.49).

Preparation—From the 21-acetate of 16α,17α-epoxy-3β,21-dihydroxypregn-5-en-20-one (available by synthesis from naturally occurring sapogenins such as diosgenin). Treatment of this pregnene with HF and *N*-bromoacetamide, followed by chromic acid oxidation and then treatment with HBr in acetic acid gives the Δ^4-16β-bromo-6α-fluoro derivative. This latter compound on refluxing with potassium acetate in acetic acid and then saponifying with sodium carbonate yields the 6α-fluoro-16α,17α-dihydroxy compound which when incubated with minced, defatted bovine adrenals adds an 11β-hydroxyl group. From the 16,21-diacetate, with dimethylformamide and methanesulfonyl chloride, the 4,9-diene is synthesized, which is converted to the 9β,11β-epoxide and then to the 9α-fluoro-11β-hydroxy compound in a manner similar to that for *Betamethasone* (page 962). Oxidation of this product with selenium dioxide yields the 1,4-diene (fluocinolone) which on reaction with acetone and perchloric acid yields the acetonide.

Description—White, crystalline powder that is odorless; stable in light; melts at about 270°, with decomposition.

Solubility—1 g in >1000 mL water, 45 mL alcohol, 25 mL chloroform, 350 mL ether.

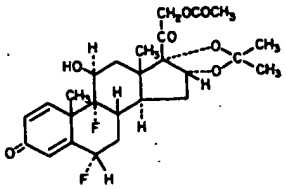
Uses—A glucocorticoid with potent anti-inflammatory and metabolic actions and negligible mineralocorticoid actions (see page 958). It is employed topically in the treatment of various *dermatoses*. In resistant nummular dermatitis, psoriasis, or chronic neurodermatitis it is usually used under occlusive dressings. Even in instances in which nearly the whole body has been covered by a cream containing the corticoid, evidences of systemic side effects are rare. However, folliculitis or striae is a frequent complication, especially if occlusive dressings are used. Topical fluocinolone is contraindicated in the presence of tuberculosis, fungal infections, and most viral lesions of the skin (vaccinia, varicella, herpes simplex, etc.). Neomycin is often included in topical preparations of fluocinolone acetonide to suppress infections secondary to the inflammatory process or which result from the use of the glucocorticoid.

Dose—Topical, to the *skin*, *adult*, as 0.01 to 0.2% cream, 0.025% ointment, or 0.01% solution, applied 2 to 4 times a day or under an occlusive dressing; *children*, as 0.01% cream or solution once or twice a day or as 0.025 to 0.2% cream or 0.025% ointment once a day.

Dosage Forms—Cream: 0.01, 0.025, and 0.2%; Ointment: 0.025%. Topical Solution: 0.01%.

Fluocinonide

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6,9-difluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-, Fluocinolide;
Lidex, Topsyn (*Syntex*)



6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone, 21-acetate [356-12-7] $C_{26}H_{32}F_2O_7$ (494.53).

Preparation—*Fluocinolone Acetonide* (this page) is esterified with acetic anhydride in the presence of pyridine. US Pats 3,126,372 and 3,592,930.

Description—White to creamy white, odorless, crystalline powder, stable in light, air, and at room temperature; melts, within a range of 3°, at about 300°, with decomposition.

Solubility—Insoluble in water; soluble 1 g in 70 mL of alcohol, 10 mL of acetone, or 10 mL of chloroform.

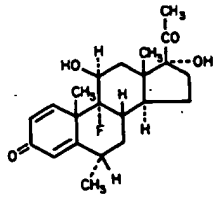
Uses—A glucocorticoid used only topically for its anti-inflammatory effects in glucocorticoid-responsive *dermatoses* (see introduction in this section). Systemic side effects are infrequent, but the local side effects are those of other glucocorticoids.

Dose—*Topical*, as 0.05% cream, gel, or ointment, applied 3 or 4 times a day for *adults* or once a day for *children*.

Dosage Forms—Cream: 0.05%; Gel: 0.05%; Ointment: 0.05%

Fluorometholone

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-6-methyl-, (6α,11β)-, Oxylone (*Upjohn*)



9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione [426-13-1] $C_{22}H_{29}FO_4$ (376.47).

Preparation—6α-Methyl-9α-fluoroprednisolone is esterified with *p*-toluenesulfonyl chloride to give the 21-*p*-toluenesulfonate. This is treated with sodium iodide in acetone solution to form the corresponding 21-iodo compound which is then reduced with sodium bisulfite to fluorometholone. US Pats 2,852,511 and 2,867,637.

Description—White to yellowish-white, odorless, crystalline powder, melts at about 280°, with decomposition.

Solubility—1 g in >10,000 mL water, 200 mL alcohol, 2200 mL chloroform, >10,000 mL ether.

Uses—A glucocorticoid with typical actions and side effects (see introduction in this section). By the oral route it is equipotent to cortisol, but by topical administration it is 40 times as potent. Consequently, it is used for topical treatment of glucocorticoid-responsive

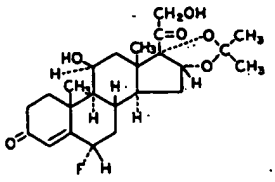
dermatoses and *ocular inflammations*. Under occlusive dressings it is particularly used to treat resistant nummular dermatitis, psoriasis, and chronic neurodermatitis.

Dose—*Topical*, to the *skin*, *as* 0.025% cream, 1 to 3 times a day for *adults* and once a day for *children*; to the *conjunctiva*, *adults* and *children*, 1 drop of 0.1% ophthalmic suspension 2 to 4 times a day.

Dosage Forms—Cream: 0.025%; Ophthalmic Suspension: 0.1%.

Flurandrenolide

Pregn-4-ene-3,20-dione, 6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-, Flurandrenolone Acetonide; Cordran (*Lilly*)



6α-Fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione, cyclic 16,17-acetal with acetone [1524-88-5] $C_{24}H_{33}FO_6$ (436.52).

Preparation—Flurandrenolone (6α-fluoro-16α-hydroxycortisol) is condensed with acetone by treating its solution in acetone with 70% perchloric acid. US Pat 3,126,375.

Description—White to off-white, fluffy, odorless, crystalline powder.

Solubility—1 g in 72 mL alcohol, 10 mL chloroform; practically insoluble in water, ether.

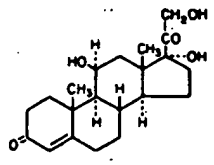
Uses—A glucocorticoid that has high potency topically but low potency systemically because of rapid destruction in the liver. Consequently, its use is limited to management of *glucocorticoid-responsive dermatologic disorders*. Under occlusive dressings it is used especially to treat nummular dermatitis, psoriasis, and chronic neurodermatitis. Local side effects are uncommon but are typical of drugs of this class (see the introduction in this section). Neomycin is included in some topical preparations of flurandrenolide to suppress infections secondary to the inflammatory process or to the use of the glucocorticoid.

Dose—*Topical*, to the *skin*, *adult*, as 0.025 or 0.05% cream or ointment or 0.05% lotion 2 or 3 times a day or as a tape (plaster) containing 4 μg/cm² 1 or 2 times a day. *Children*, as 0.025% cream or ointment 1 or 2 times a day or as 0.05% cream, lotion, or ointment, or as tape containing 4 μg/cm² once a day.

Dosage Forms—Cream: 0.025 and 0.05%; Lotion: 0.05%; Ointment: 0.025 and 0.05%; Tape: 4 μg/cm².

Hydrocortisone

Pregn-4-ene-3,20-dione, 11,17,21-trihydroxy-, (11β)-, Compound F; Reichstein's "Substance M"; Cortef (*Upjohn*); Cortril (*Pfizer*); Hydrocortone (*MSD*); *Various Mfrs*



Cortisol [50-23-7] $C_{21}H_{30}O_5$ (362.46).

Preparation—The most attractive commercial synthesis involves the oxidation of 17α,21-dihydroxypregn-4-ene-3,20-dione, which is readily obtainable from diosgenin. Microbiological hydroxylation at the 11β-position is affected on the diacetate of the above compound employing organisms of the *Rhizopus*, *Aspergillus* or *Streptomyces* species. Saponification then yields hydrocortisone.

Description—White to practically white, odorless, crystalline powder; melts at about 215°, with decomposition.

Solubility—1 g in 40 mL alcohol; very slightly soluble in water and ether; slightly soluble in chloroform.

Uses—The principal natural glucocorticoid in man and thus the prototype of all glucocorticoids (for actions, uses, and side effects of glucocorticoids, see the general statement in this section). Systemic side effects can result from topical application. Allergic bronchospasm after hydrocortisone in asthmatics has been reported. The plasma half-life is 1½ to 3 hr.

Some topical preparations include neomycin and/or other antibiotics to suppress emergence of infections.

Dose—*Adult*, *oral*, for *replacement*, 25 to 50 mg/day, ⅔ of which is to be taken in the morning and ⅓ in the afternoon; for *anti-inflammatory* use, 20 to 240 mg a day in 3 or 4 divided doses. *Topical*, to the *skin*, as 0.125 to 2.5% cream, 0.25 to 1% gel, or 0.25 to 2.5% lotion 3 or 4 times a day; to the *scalp*, as 0.5% aerosol spray once a day initially and later decreased to 1 to 3 times a week; to the *eye*, as 0.2% ophthalmic solution, as 1% ophthalmic suspension containing antibiotics, or 0.5 to 1.5% ointment containing antibiotics every hour during the day and every 2 hours at night; in the *ear* as 1% otic ointment, solution, or suspension containing antibiotics; *rectal*, as 10-mg suppository once a day; *enema*, 100 mg once a day; *intravaginal*, 10 mg; *intrasynovial* or *intralesional*, 10 to 50 mg; to *oral mucosa*, as a 0.5% paste 2 or 3 times a day after meals and at bedtime. *Children*, *oral*, for *replacement*, 0.56 mg per kg of body weight (or 16 mg per m² of body surface); for *anti-inflammatory* use, 2 to 8 mg per kg of body weight (or 60 to 240 mg per m² of body surface); *topical*, to the *skin*, as 0.125 to 1% cream or lotion or 0.25 to 1% gel or ointment 1 or 2 times a day or 2.5% cream or ointment once a day; to the *scalp*, adult dose.

Dosage Forms—Cream: 0.5, 1, and 2.5%; Dental paste: 0.5%; Enema: 100 mg/60 mL; Gel: 0.25 and 1%; Lotion: 0.25, 0.5, and 1%; Ointment: 0.5, 1, and 2.5%; Ophthalmic Solution: 0.2%; Sterile Suspension: 50 mg/mL; Tablets: 5, 10, and 20 mg; Topical Aerosol: 0.5%.

Hydrocortisone Acetate

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-; Hydrocortisone 21-Acetate; Cortef Acetate (*Upjohn*); Cortril Acetate (*Pfizer*); Hydrocortone Acetate (*MSD*)

Cortisol 21-acetate [50-03-3] $C_{23}H_{32}O_6$ (404.50).

Preparation—Hydrocortisone is esterified with acetic anhydride to give the 21-acetate.

Description—White to practically white, odorless, crystalline powder; melts at about 220°, with decomposition.

Solubility—Insoluble in water; 1 g in 230 mL alcohol, 200 mL chloroform.

Uses—Has the actions of *Hydrocortisone*, to which it is converted in the body. However, it is not used for systemic therapy. It is used topically in the treatment of glucocorticoid-sensitive dermatoses, anorectal inflammations, inflammatory conditions of the eye, and intra-articularly in the treatment of arthritides. Systemic effects can result from local application.

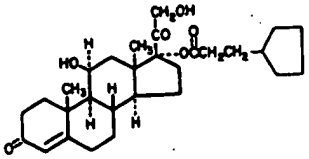
The inclusion of neomycin or antifungal drugs in lotions and creams containing hydrocortisone acetate is for the purpose of protecting against bacterial or fungal infections that might be favored by the suppression of the inflammatory response and of clearing up infections secondary to the inflammatory condition.

Dose—*Intra-articular*, *intralesional*, or *soft-tissue injection*, *adult*, 5 to 75 mg repeated at 1- to 3-week intervals, if necessary. *Topical, to the skin*, *adult*, as 0.5 to 1% cream or 0.5 to 5% lotion 3 or 4 times a day or 1 to 2.5% ointment 1 to 4 times a day; *children*, as 0.5 to 1% cream or lotion or 1% ointment 1 or 2 times a day or as a 2.5 to 5% lotion or 2.5% ointment once a day. *Topical*, to the *eye*, *adults* and *children*, a thin strip of 0.5 to 1.5% ointment or 1 or 2 drops of a 0.2% solution or 2.5% suspension every hour during the day and every 2 hours at night for the solution and 3 or 4 times a day for the other forms; *rectal*, *adult* as 1.0% aerosol foam 1 to 3 times a day for 3 weeks, after which dosage is diminished every other day, or as suppository 15 to 25 mg twice a day for 2 weeks, except 3 times a day or 30 to 50 mg twice a day in severe proctitis.

Dosage Forms—Aerosol Foam: 1%; Cream: 0.5% or 0.5% of hydrocortisone equivalent, except 1% in certain combinations; Lotion: 0.5% of hydrocortisone equivalent; Ointment: 1 and 2.5%; Ophthalmic Ointment: 0.5 and 1.5%; Ophthalmic Suspension: 2.5%; Sterile Suspension: 25 and 50 mg/mL; Suppositories: 15 and 25 mg, except 10 mg in some combinations.

Hydrocortisone Cypionate

Pregn-4-ene-3,20-dione, 21-(3-cyclopentyl-1-oxopropoxy)-11,17-dihydroxy-, (11β)-, Cortisol Cypionate; Cortef Fluid (*Upjohn*)



Cortisol 21-cyclopentanepropionate [508-99-6] $C_{29}H_{42}O_6$ (486.65).

Preparation—*Hydrocortisone* is esterified by treatment with cyclopentanepropionyl chloride in the presence of pyridine.

Description—White to practically white, crystalline powder; odorless or has a slight odor.

Solubility—Insoluble in water; slightly soluble in ether; soluble in alcohol; very soluble in chloroform.

Uses—Actions and systemic uses are those of *Hydrocortisone*, to which it is converted in the body. Because of its low solubility, its absorption from the gastrointestinal tract is slower than that of hydrocortisone; also, its taste is more pleasant than that of hydrocortisone, so that it is used for oral therapy.

Dose—*Oral, adult*, the equivalent of **20 to 240 mg** of hydrocortisone daily, as a single dose or in divided doses; *children*, for *replacement*, the equivalent of **0.56 mg per kg** of body weight (or **16 mg per m²** of body surface) of hydrocortisone a day, or, for *anti-inflammatory* use, the equivalent of **2 to 8 mg per kg** of body weight (or **60 to 240 mg per m²** of body surface) a day.

Dosage Form—Oral Suspension: the equivalent of 10 mg of hydrocortisone/5 mL.

Hydrocortisone Sodium Phosphate

Pregn-4-ene-3,20-dione, 11,17-dihydroxy-21-(phosphonooxy)-, disodium salt, (11β)-, Cortisol Sodium Phosphate; Hydrocortone Phosphate (*MSD*); Corphos (*Cooper*)

Cortisol 21-(disodium phosphate) [6000-74-4] $C_{21}H_{29}Na_2O_8P$ (486.41).

For the structure of the base, see *Hydrocortisone*.

Preparation—From hydrocortisone by a method similar to that used for *Dexamethasone Sodium Phosphate*. US Pat 2,870,177.

Description—White to light-yellow, odorless or practically odorless, bitter-tasting powder; relatively stable in light and heat and very hygroscopic; pH (1% solution) 7.5 to 8.5.

Solubility—1 g in about 1.5 mL water; slightly soluble in alcohol; practically insoluble in chloroform, dioxane, and ether.

Uses—Has the same actions and uses as *Hydrocortisone*, to which it is converted in the body. However, the phosphate is quite soluble and hence has special usefulness as a parenteral form of cortisol in emergency situations in which a rapid response is essential or when oral medication cannot be tolerated.

Dose—The doses below are stated in terms of the hydrocortisone equivalents. *Intramuscular, intravenous*, or *subcutaneous, adult*, **15 to 240 mg** a day until the condition responds, after which the dosage is gradually decreased; in *acute adrenal insufficiency*, **100 mg** is given *intravenously* followed by **100 mg** every 8 hr until the patient is out of danger. *Children, intramuscular*, for *replacement*, **560 μg/kg** or **16 mg/m²** of body surface a day in 3 divided doses every third day *or* **186 to 280 μg/kg** or **5.33 to 8 mg/m²** once a day; for *disease*, **660 μg to 4 mg/kg** or **20 to 120 mg/m²** every 12 to 24 hr; *intravenous*, for *acute adrenal insufficiency, infants*, **1 to 2 mg/kg** followed by **25 to 150 g/kg/day** and *older children*, **1 to 2 mg/kg** followed by **150 to 250 μg/kg/day** in divided doses.

Dosage Form—Injection: the equivalent of 50 mg of hydrocortisone/mL.

Hydrocortisone Sodium Succinate

Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, monosodium salt, (11β)-, A-hydroCort (*Abbott*); Solu-Cortef (*Upjohn*)

Cortisol 21-(sodium succinate) [125-04-2] $C_{25}H_{33}NaO_8$ (484.52).

Preparation—Hydroxycortisone 21-(hydrogen succinate) is first prepared by reacting hydrocortisone with succinic anhydride dissolved in pyridine. When the reaction is complete, the mixture is added to cold, dilute HCl whereupon the acid ester precipitates. It is collected, washed with water, dried, and purified by recrystallizing from acetone. The sodium salt is then prepared by neutralizing the acid with dilute NaOH solution followed by drying the solution from the frozen state.

Description—White or nearly white, odorless, hygroscopic, amorphous solid.

Solubility—Very soluble in water and alcohol; insoluble in chloroform; very slightly soluble in acetone.

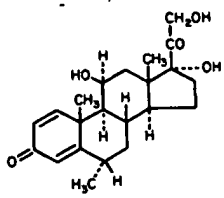
Uses—The actions and uses are the same as those of *Hydrocortisone*, into which it is converted in the body. However the sodium succinate derivative is highly soluble and hence is a desirable form for infusion concentrates and for intravenous or intramuscular administration when intense rapid action is desired. It is intended only for systemic short-term emergency therapy.

Dose—The same as that of *Hydrocortisone Sodium Phosphate*, above.

Dosage Forms—For Injection: the equivalent of 100, 250, and 500 mg and 1 g of hydrocortisone.

Methylprednisolone

Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-6-methyl-, (6α,11β)-, Medrol (*Upjohn*)



11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione [83-43-2] $C_{22}H_{30}O_5$ (374.48).

Preparation—*Progesterone* (page 993) is converted to the 6α-methyl derivative in the same manner as indicated in the synthesis of *Medroxyprogesterone Acetate* (page 992). Incubation of the 6α-methyl compound with an Ascomycete, such as *Pestalotia*, forms the 11α-hydroxy derivative which is oxidized to the 3,11-dikeio compound with chromic acid. Further treatment with ethyl oxalate followed by bromination, rearrangement with sodium methoxide and debromination with zinc dust gives the methyl ester of the 4,17(20)-diene-21-carboxylate. With pyrrolidine, lithium aluminum hydride reduction and treatment with alkali, the 11β,21-dihydroxy-4,17(20)-diene is formed which is converted to the 21-acetate and then oxidatively hydroxylated to 6α-methylhydrocortisone acetate. Saponification, followed by dehydrogenation with *Septomyxa affinis* gives the 1,4,17(20)-triene, which is again converted to the 21-acetate, oxidatively hydroxylated to yield the 17α-hydroxy derivative and saponified to give methylprednisolone.

Description—White to practically white, odorless, crystalline powder; melts at about 240° with some decomposition.

Solubility—1 g in 10,000 mL water, 100 mL alcohol, 800 mL chloroform, 800 mL ether.

Uses—A glucocorticoid with actions, uses, and side effects typical of drugs of this class (see introduction in this section). It induces considerably less retention of sodium and water than the parent prednisolone. Because methylprednisolone possesses only weak mineralocorticoid activity, it is not employed in the management of acute adrenal insufficiency. The plasma half-life is 3 to 4 hours.

Dose—*Oral, adult*, 4 to 48 mg a day; *children*, for *replacement*, **117 μg to 1.7 mg per kg** of body weight (or **3.3 to 50 mg per m²** of body surface) a day; for *disease*, **417 μg to 1.67 mg/kg** (or **1.25 to 50 mg/m²** of body surface) a day in 3 divided doses.

Dosage Forms—Tablets: 2, 4, 8, 16, 24, and 32 mg.

Methylprednisolone Acetate

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-6-methyl-, (6α,11β)-, Methylprednisolone 21-Acetate; Depo-Medrol and Medrol Acetate; (*Upjohn*)

11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-acetate [53-36-1] $C_{24}H_{32}O_6$ (416.51).

For the structure of the base, see *Methylprednisolone*.

Preparation—The 21-acetate compound obtained in the synthesis of *Methylprednisolone*, just prior to the final saponification.

Description—White or practically white, odorless, crystalline powder; melts at about 225° with some decomposition.

Solubility—1 g in 1500 mL water, 400 mL alcohol, 250 mL chloroform, 1500 mL ether.

Uses—Converted in the body to *Methylprednisolone*, over which it has no advantage in systemic therapy; thus the acetate is employed principally for local therapy. As a suspension it may be given intra-articularly or topically. Topical uses and adverse effects are discussed in the introduction in this section. Methylprednisolone acetate is combined with neomycin in some topical preparations.

Dose—*Intramuscular, adult, initially* 4 to 120 mg repeated at one-day to two-week intervals, if necessary; *children*, for *replacement*, 117 μg/kg (or 3.33 mg/m² of body surface) in 3 divided doses in one day, repeated every third day, *or* 39 to 58 μg/kg (or 1.11 to 1.66 mg/m²) once a day; for *disease*, 139 to 835 μg/kg (or 4.16 to 25 mg/m² every 12 to 24 hr. *Intra-articular, intralesional* or into *soft tissue*, 4 to 80 mg. *Topical*, as 0.25% or 1% cream, applied 1 to 4 times a day on *adults* and 1 or 2 times a day on *children*. *Rectal, adult*, 40 mg as retention enema 3 to 7 times a week for 2 or more weeks; *children*, 500 μg to 1 mg/kg (or 15 to 30 mg/m²) every 1 or 2 days for 2 or more weeks. *Topical*, to the *conjunctiva, as* 0.1% ophthalmic ointment 1 to 3 times a day.

Dosage Forms—Cream: 0.25 and 1%; for Enema: 40 mg; Ophthalmic Ointment: 0.1%; Sterile Suspension: 40 and 80 mg/1 mL, 100, 200 and 400 mg/5 mL, and 400 mg/10 mL.

Methylprednisolone Sodium Succinate

Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, monosodium salt, (6α,11β)-, Methylprednisolone 21-(Sodium Succinate); Solu-Medrol (*Upjohn*); A-Methapred (*Abbott*)

11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(sodium succinate) [2375-03-3] $C_{26}H_{33}NaO_8$ (496.53).

For the structure of the base, see *Methylprednisolone*.

Preparation—*Methylprednisolone* is treated with succinic anhydride in pyridine and added to dilute HCl to precipitate the hemisuccinate which is neutralized with NaOH in aqueous acetone solution and the solvent removed by lyophilization.

Description—White, or nearly white, odorless, hygroscopic, amorphous solid.

Solubility—1 g in 1.5 mL water, 12 mL alcohol, >10,000 mL chloroform, >10,000 mL ether.

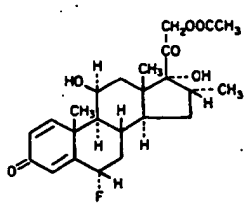
Uses—The actions are the same as those of *Methylprednisolone*, into which it is converted in the body; for actions, uses, and adverse effects of glucocorticoids see the introduction in this section. The solubility of methylprednisolone sodium succinate makes its use advantageous for parenteral and intra-articular administration when rapid and intense action is desired. It is used systemically only for short-term treatment.

Dose (as methylprednisolone equivalent)—*Intravenous* or *intramuscular, adult*, 10 to 40 mg as needed, except 5 mg per kg of body weight as a *bolus* every 4 hr in *shock*. *Intramuscular, children*, for *replacement* and *disease* therapy, see *Methylprednisolone Acetate*. *Intra-articular* or *intralesional*, 4 to 80 mg, as needed.

Dosage Forms—For Injection: 40, 125, 500 mg, and 1 g (as methylprednisolone).

Paramethasone Acetate

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6-fluoro-11,17-dihydroxy-16-methyl-, (6α,11β,16α)-, Haldrone (*Lilly*); Monocortone (*Syntex*)



6α-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate [1597-82-6] $C_{24}H_{31}FO_6$ (434.50).

Preparation—3-Hydroxy-16α-methylpregn-5-en-20-one acetate (16α-methylpregnenolone acetate) is subjected to a series of standard reactions to form 6α-fluoro-17,21-dihydroxy-16α-methylpregn-4-ene-3,20-dione, then hydroxylated at the 11β-position by incubation with bovine adrenal glands and converted to the 21-acetate. Dehydrogenation with selenium dioxide in the presence of pyridine completes the synthesis with creation of the 1,2-double bond.

Description—Fluffy, practically white, odorless, crystalline powder; exposure to light should be avoided. It melts at about 240° with decomposition.

Solubility—Very soluble in alcohol; soluble in acetone, chloroform, ether, and methanol; insoluble in water.

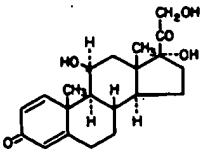
Uses—A glucocorticoid with actions, uses, and side effects typical of drugs of this class (see introduction in this section), except that it is almost devoid of mineralocorticoid side effects, although there may be occasional edema and rare hypertension. It is 10 times as potent as cortisone, but potency does not confer any particular advantages. Increased appetite and weight gain occur in only about ⅓ of patients. The catabolic effects, such as protein depletion and osteoporosis, are only moderate with low to moderate doses of the drug.

Dose—*Oral, adult, initially* 2 to 8 mg a day in mild disorders, 8 to 12 mg a day in moderately severe disorders, and 20 to 40 mg a day in life-threatening illness, the dose being gradually reduced once the condition improves. *Children*, 58 to 800 μg per kg of body weight (or 1.67 to 25 mg per m² of body surface) a day.

Dosage Forms—Tablets: 1 and 2 mg.

Prednisolone

Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-, (11β)-, (*Various Mfrs*)



11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione [50-24-8] $C_{21}H_{28}O_5$ (360.45); *sesquihydrate* [52438-85-4] (387.47); anhydrous or contains one and one-half molecules of water of hydration.

Preparation—From hydrocortisone by a microbiologic process utilizing *Corynebacterium simplex* which selectively dehydrogenates cortisol at the 1 and 2 positions.

Description—White to practically white, odorless, crystalline powder; melts at about 235°, with some decomposition.

Solubility—1 g in 30 mL alcohol, 180 mL chloroform; very slightly soluble in water.

Uses—A glucocorticoid with the actions, uses, and side effects typical of drugs of this class (see the introduction in this section). It is 4 times as potent as hydrocortisone but relatively somewhat weaker than hydrocortisone as a mineralocorticoid although sodium retention and potassium depletion can occur. The plasma half-life is about 3 hr. Except for its higher solubility, it may be considered equivalent to prednisone; it is the biologically active metabolite of prednisone.

Dose—*Oral, adult, initially* usually 5 to 60 mg but may be as high as 250 mg a day until a response occurs, when the dose is gradually diminished to the smallest effective maintenance dose; *children*, for *replacement*, 140 μg/kg (or 4 mg/m² of body surface) a day in 3 divided doses, or, for *disease*, 500 μg to 2 mg/kg (or 15 to 60 mg/m²) a day in 3 divided doses. *Topical*, to the *skin*, as a 0.5% cream 3 or 4 times a day for adults and 1 or 2 times a day for children.

Dosage Forms—Cream: 0.5%; Tablets: 1 and 5 mg.

Prednisolone Acetate

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-, Prednisolone 21-Acetate; Meticortelone (*Schering*); Sterane (*Pfizer*); *Various Mfrs*

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-acetate [52-21-1] $C_{23}H_{30}O_6$ (402.49).

Preparation—From *Prednisolone* by reaction with acetic anhydride.

Description—White to practically white, odorless, crystalline powder; melts at about 235° with some decomposition.
Solubility—1 g in 120 mL alcohol; practically insoluble in water; slightly soluble in chloroform and acetone.

Uses—The actions and uses are the same as those of *Prednisolone*, into which it is converted in the body. The acetate is relatively nonirritating to the tissues and hence is suitable for intramuscular or local injection; esterification also prolongs absorption. It may be used particularly in those situations in which oral prednisolone is not feasible, but there are no contraindications to substitution of the parenteral prednisolone acetate for oral prednisolone for any purpose.
Dose—*Intramuscular, adult, initially* 20 to 60 mg a day in 4 divided doses until a satisfactory response occurs, then a gradual reduction to a minimal *maintenance* dose, which usually is in the range of 5 to 20 mg a day; *children*, for *replacement*, 140 μg/kg (or 4 mg/m²) of body surface) every third day, and, for *disease*, 166 μg to 1 mg/kg (or 5 to 30 mg/m²) every 12 to 24 hr. *Intralesional*, or *soft-tissue injection*, 4 to 60 mg every 1 to 4 weeks. *Topical*, to the *eyes*, as 0.125 to 1% ophthalmic suspension or 0.5% ointment; to the *skin*, as a 0.5% ointment (containing 0.5% neomycin).
Dosage Forms—Ointment: 0.5% (with 0.5% neomycin); Ophthalmic Ointment: 0.25 and 0.5%; Ophthalmic Suspension: 0.125, 0.25, and 1%; Sterile Suspension: 25, 40, 50, 80, and 100 mg/mL.

Prednisolone Sodium Phosphate

Pregna-1,4-diene-3,20-dione, 11,17-dihydroxy-21-(phosphonooxy)-, disodium salt, (11β)-, Prednisolone 21-(Disodium Phosphate); Hydeltrasol (*MSD*)

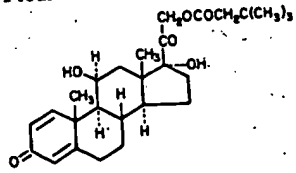
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(disodium phosphate) [125-02-0] $C_{21}H_{27}Na_2O_8P$ (484.39).
Preparation—From *Prednisolone* by a method similar to that used for *Dexamethasone Sodium Phosphate*.

Description—White or slightly yellow, friable granules or powder; odorless or has a slight odor; slightly hygroscopic.
Solubility—1 g in 4 mL water and 13 mL methanol; slightly soluble in alcohol and chloroform; very slightly soluble in acetone and dioxane.

Uses—A soluble form of *Prednisolone*, into which it is converted in the body. It is employed parenterally in emergency situations in which an intense glucocorticoid action is required. Since absorption by the intramuscular route is quite rapid, the intravenous route is infrequently employed. The high solubility of the drug also lends itself well to intrasynovial injection in the treatment of arthritides and bursitides and to local injection for inflammatory cysts and soft-tissue inflammations. It is also employed in the local treatment of a number of inflammatory eye diseases and for inflammatory and pruritic dermatoses, bites, and burns.
Dose—Doses are stated in terms of the equivalents of *Prednisolone Phosphate*. *Intravenous* or *intramuscular, adult*, 40 to 60 mg of the equivalent of prednisolone phosphate a day, except up to 2 g a day intravenously in bacteremic shock. *Intramuscular, children*, for *replacement*, 140 μg/kg (or 4 mg/m² of body surface) in 1 day in 3 divided doses every third day, *or*, 46 to 70 μg/kg (or 1.33 to 2 mg/m²) per day; for *disease*, 166 μg to 1 mg (or 5 to 30 mg/m²) every 12 to 24 hr. *Intra-articular, intralesional*, or *soft-tissue injection*, 2 to 30 mg every 3 to 5 days to 2 to 3 weeks. *Topical*, to the *conjunctiva*, a thin strip of 0.25% ointment of prednisolone phosphate equivalent 3 or 4 times a day or 1 drop of 0.113 to 0.9% ophthalmic solution, with or without antibacterial drugs, 4 to 6 times a day.
Dosage Forms (as equivalent of prednisolone phosphate)—Injection: 20 mg/mL; Ophthalmic Ointment: 0.25%; Ophthalmic Solution: 0.125, 0.5, and 1%; Ophthalmic/Otic Solution: 0.5%.

Prednisolone Tebutat

Pregna-1,4-diene-3,20-dione, 11,17-dihydroxy-21-[(3,3-dimethyl-1-oxobutyl)oxy]-, (11β)-, Prednisolone *tert*-butylacetate; Hydeltra T.B.A. (*MSD*) Prednalone T.B.A. (*O'Neal, Jones & Feldman*)



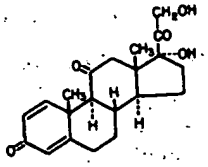
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(3,3-dimethylbutyrate) [7681-14-3] $C_{27}H_{38}O_6$ (458.59); *monohydrate* (476.61).
Preparation—From *Prednisolone* by esterification of the 21-hydroxyl group with 3,3-dimethylbutyryl chloride.

Description—White to slightly yellow powder; odorless or has not more than a moderate, characteristic odor; melts between 240° and 250°.
Solubility—Very slightly soluble in water; freely soluble in chloroform; soluble in acetone; sparingly soluble in alcohol.

Uses—Converted in the body to *Prednisolone*. At present, its use is confined to local injection into inflamed joints, tendons, and bursae or into soft-tissue lesions. Its low solubility results in a repository action, with an onset of action of 1 to 2 days and a duration of 2 to 3 weeks. Temporary local discomfort may follow injection.
Dose—*Intra-articular, intrasynovial, intralesional*, or *soft-tissue injection*, 4 to 40 mg once every 2 or 3 weeks.
Dosage Form—Sterile Suspension: 20 mg/mL.

Prednisone

Pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-, (*Various Mfrs*)



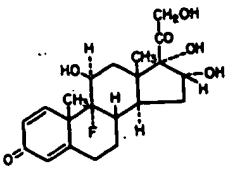
17,21-Dihydroxypregna-1,4-diene-3,11,20-trione [53-03-2] $C_{21}H_{26}O_5$ (358.43).
Preparation—As described for *Prednisolone* except that cortisone is used instead of hydrocortisone.

Description—White to practically white, odorless, crystalline powder; melts at about 230°, with some decomposition.
Solubility—1 g in 150 mL alcohol, 200 mL chloroform; very slightly soluble in water.

Uses—A dehydrogenated derivative of cortisone with actions, uses, and side effects typical of glucocorticoids (see introduction in this section). It has 3 to 5 times the glucocorticoid activity of hydrocortisone but somewhat less of mineralocorticoid activity, although sodium retention and potassium depletion may occur. It cannot be used alone for replacement therapy in adrenal insufficiency. Prednisone is the glucocorticoid predominantly used in *cancer chemotherapy*, always in combination with other drugs. It is also the glucocorticoid most used in the treatment of acute exacerbations of *multiple sclerosis*. In pediatrics it is widely used to treat *nephrosis, rheumatic carditis, leukemias, other tumors*, and *tuberculosis*.
The plasma half-life is 3 to 5 hr, but the effects last 12 to 36 hr.
Dose—*Oral, adult, initially* usually 5 to 60 mg but may be as high as 250 mg a day until a satisfactory response occurs, when the dose is gradually diminished to the smallest effective *maintenance* dose, usually 10 to 20 mg a day; for acute exacerbations of *multiple sclerosis*, 200 mg/day for 1 week, then 80 mg every other day for 1 month. *Children*, 35 to 500 μg per kg of body weight (or 1 to 15 mg per m² of body surface), 4 times a day. However, dose regimens vary greatly with the use; for the details, see the USP DI, the AMA Drug Evaluations, the Physicians Desk Reference, or the package literature.
Dosage Forms—Syrup: 5 mg/5 mL; Tablets: 1, 2.5, 5, 10, 20, 25, and 50 mg.

Triamcinolone

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,16,17,21-tetrahydroxy-, (11β,16α)-, Aristocort (*Lederle*); Kenacort (*Squibb*)



9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, [124-94-7] $C_{21}H_{27}FO_6$ (394.44).

Preparation—From hydrocortisone acetate via the 3,20-bisketal by treatment with thionyl chloride, refluxing with potassium hydroxide and acetylation to give 21-acetoxy-4,9,11(16)-pregnatriene-3,20-dione. Oxidation with osmium tetroxide to the 16α,17α-dihydroxy derivative and subsequent insertion of the 9α-fluoro and 11β-hydroxy groups as indicated for *Betamethasone* (page 962), gives a product lacking only a double bond at the 1-position. This latter step is accomplished by incubation with *Nocardia corallina*, followed by saponification of the acetate to yield triamcinolone. Alternatively, the compound can be made from *Fludrocortisone* by enzymatically inserting the 16α-hydroxyl group and dehydrogenating as above at the 1,2-position.

Description—Fine, white or practically white, crystalline powder having not more than a slight odor; its polymorphic forms and/or solvates melt between 248° and 250°; 260° 263°, 269° and 271°.

Solubility—1 g in about 5000 mL water, 70 mL propylene glycol, and less than 20 mL dimethyl sulfoxide; slightly soluble in alcohol and chloroform.

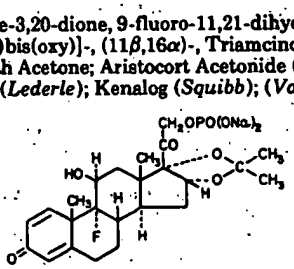
Uses—A glucocorticoid with actions, uses, and side effects typical of drugs of this class (see introduction in this section). As a glucocorticoid it is 7 to 13 times more potent than hydrocortisone. It has been claimed that therapeutic doses of triamcinolone are nearly devoid of mineralocorticoid and other side effects of hydrocortisone but the mineralocorticoid actions vary from patient to patient. It appears that the drug may induce naturesis, negative sodium balance with weight loss in most patients (along with headache, dizziness, and fatigue), and sodium retention with weight gain, moon face, etc. in others. Nearly every side effect seen with hydrocortisone has been observed with triamcinolone, but the relative frequencies are less; however, it does not increase appetite and thus differs from other glucocorticoids. By the oral route, more triamcinolone survives the first pass through the liver than does hydrocortisone, and blood levels are somewhat more predictable. The plasma half-life is about 5 hours.

Dose—*Oral, adult*, in *adrenal insufficiency*, 4 to 12 mg a day in single or divided doses (along with a mineralocorticoid); in *disease*, 4 to 48 mg a day. *Children*, in *adrenal insufficiency*, 117 μg per kg of body weight (or 3.3 mg per m² of body surface) a day; in *disease*, 416 μg to 1.7 mg per kg of body weight (or 12.5 to 50 mg per m² of body surface) a day.

Dosage Forms—Tablets: 1, 2, 4, 8, and 16 mg.

Triamcinolone Acetonide

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-, Triamcinolone 16,17-Cyclic Acetal with Acetone; Aristocort Acetonide (*Lederle*); Aristoderm (*Lederle*); Kenalog (*Squibb*); (*Various Mfrs*)



9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone [76-25-5] $C_{24}H_{31}FO_5$ (434.50).

Preparation—*Triamcinolone* is treated with acetone and perchloric acid followed by neutralization and vacuum concentration.

Description—White to cream-colored, crystalline powder having not more than a slight odor; melts between 290° and 294°.

Solubility—Practically insoluble in water; very soluble in dehydrated alcohol, chloroform, and methanol; sparingly soluble in acetone and ethyl acetate; slightly soluble in alcohol.

Uses—Triamcinolone acetonide is a high-potency glucocorticoid with the actions, uses, and side effects typical of that class of drugs (see introduction in this section). It has a higher lipid-water distribution coefficient than triamcinolone and is thus more suitable for topical use.

Dose—*Topical*, to the *skin*, *adult*, as 0.025, 0.1, or 0.5% cream or ointment, 0.1% aerosol foam or lotion, 0.1% gel, 0.025 or 0.1% lotion, or 0.015% aerosol solution, 2 to 4 times a day; *children*, as 0.025% cream, lotion, or ointment, or 0.015% aerosol solution 1 or 2 times a day, or 0.1 or 0.5% cream or ointment or 0.1% gel, lotion or aerosol lotion once a day. *Topical*, to the *oral mucous membranes*, as 0.1% paste 1 to 3 times a day (after meals or at bedtime). *Intramuscular*, 40 to 80 mg, repeated at 4-week intervals, in *adults* and 40 mg or 30 to 200 μg/kg (or 1 to 6.25 mg/m² of body surface), repeated at 7-day intervals, in *children*. *Intraarticular*, *intrabursal*, or into the *tendon sheath*, *adults* and *children*, 2.5 to 15 mg at weekly intervals or more frequently. *Intradermal* or *intralesional*, *adults*, up to 1 mg at intervals up to 1 week.

Dosage Forms—Cream: 0.025, 0.1, and 0.5%; Aerosol Foam: 0.1%; Gel: 0.1%; Aerosol Lotion: 0.1%; Lotion: 0.025 and 0.1%; Ointment: 0.025, 0.1, and 0.5%; Dental Paste: 0.1%; Aerosol Solution: 0.015%; Sterile Suspension: 10 and 40 mg/mL.

Triamcinolone Diacetate

Pregna-1,4-diene-3,20-dione, 16,21-bis(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β,16α)-, Triamcinolone 16,21-Diacetate; Aristocort Diacetate (*Lederle*); Kenacort (*Squibb*); Triamolone 40 (*O'Neal, Jones & Feldman*)

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,21-diacetate [67-78-7] $C_{25}H_{31}FO_8$ (478.51).

For the structure of the base, see *Triamcinolone*.

Preparation—By direct acetylation of triamcinolone. Among other ways, it has also been prepared from 11β,16α,17,21-tetrahydroxypregn-4-en-3,20-dione (16α-hydroxycortisone) through the following sequence of reactions: (a) microbiological oxidation with *Nocardia corallina* or *Corynebacterium simplex* to the pregna-1,4-diene analogue, (b) acetylation yielding the 16α,21-diacetate, (c) selective dehydration involving the 11-hydroxy with thionyl chloride to form the 1,4,9(11)-pregnatriene compound, (d) addition of hypobromous acid to the 9,11-double bond followed by treatment with potassium acetate in ethanol to form the 9,11-epoxy compound, and (e) rupturing of the epoxy ring with hydrogen fluoride to introduce the 9α-fluorine.

Description—Fine, white or slightly off-white crystals that have not more than a slight odor and a slight, bitter taste. Prolonged heating above 100° will convert the hydrate to the anhydrous form.

Solubility—1 g in 13 mL alcohol, 80 mL chloroform; practically insoluble in water; slightly soluble in ether.

Uses—The actions and uses are identical with those of *Triamcinolone*. However, its slight solubility is such that on injection it has reasonably prompt onset of action yet a duration of action longer than that of more soluble preparations. It also has a more agreeable taste than triamcinolone and can thus be given in liquid oral preparations.

Dose (as triamcinolone equivalent)—*Oral, adult*, for *replacement*, 4 to 12 mg per day (along with a mineralocorticoid) and, for *anti-inflammatory* use, 8 to 48 mg a day. *Children*, for *replacement*, 117 μg per kg of body weight (or 3.3 mg per m² of body surface) a day (with a mineralocorticoid) and, for *anti-inflammatory* use, 416 μg to 1.7 mg per kg of body weight (or 12.5 to 50 mg per m² of body surface) a day. *Intra-articular*, *intrasynovial*, *intralesional* or into *soft tissue*, *adult*, 3 to 48 mg. *Intramuscular*, *adult* and *children* over 6 *yrs* of age, 40 mg once a week.

Dosage Forms—Sterile Suspension: 40 mg/1 mL and 125 and 200 mg/5 mL; Syrup: 2 and 5 mg/5 mL.

Triamcinolone Hexacetonide

Pregna-1,4-diene-3,20-dione, 21-(3,3-dimethyl-1-oxobutoxy)-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-, Aristospan (*Lederle*)

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone 21-(3,3-dimethylbutyrate) [5611-51-8] $C_{30}H_{41}FO_7$ (532.65). For the structure of the base, see *Triamcinolone*.

Preparation—*Triamcinolone Acetonide* is 21-esterified by reaction with 3,3-dimethylbutyryl chloride in the presence of pyridine.

Description—White to cream-colored, crystalline powder; odorless and tasteless to slightly bitter tasting; relatively stable to light, heat, and air; decomposes at about 295°; no polymorphs have been reported.

Solubility—1 g in 167 mL methanol and less than 20 mL chloroform; practically insoluble in water.

Uses—Gradually converted to *Triamcinolone* in the body and hence has the same potential actions, uses, and side effects. At present, it is used only for injection into inflamed joints and soft-tissue lesions. It is quite insoluble and hence has a repository action.

Dose—*Intra-articular* or *intrasynovial, adult*, 2 to 20 mg, repeated at 3- or 4-week intervals, if necessary. *Intralesional* or *sublesional, adult*, up to 0.5 mg per in^2 of diseased skin.

Dosage Forms—Sterile Suspension: 20 mg/1 mL and 25 and 100 mg/5 mL.

Other Adrenal Hormones

Amcinonide [9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclopentanone, 21-acetate [51022-69-6] $C_{28}H_{35}FO_7$ (502.58)]—A topical glucocorticoid that appears to have a slightly higher topical activity and bioavailability from topical formulations than triamcinolone acetonide and betamethasone valerate. *Dose: Topical*, to the *skin*, as 0.1% cream 2 or 3 times a day in adults and once a day in children.

Betamethasone Benzoate [9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-benzoate [22298-29-9] $C_{29}H_{33}FO_6$ (496.57); Benisone (*Warner/Chilcott*); Flurobate (*Texas Pharmacal*); Uticort (*Parke-Davis*)]—A glucocorticoid with the actions of *Betamethasone* (page 962). It is used only topically for relief of inflammation in glucocorticoid-responsive dermatoses (see the general statement for topical uses and side effects). *Dose: Topical*, as 0.025% gel, cream, or lotion applied 2 to 4 times a day to adults but only once a day to children.

Betamethasone Dipropionate [9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4] $C_{28}H_{37}FO_7$ (504.59); Diprosone (*Schering-Plough*)]—The actions of this glucocorticoid are those of Betamethasone (page 962), into which it is converted; its greater lipid-solubility makes it more suitable for topical therapy. It is used only to treat glucocorticoid-responsive dermatoses. For side effects of cutaneous use see the general statement in this section. *Dose: Topical*, to the *skin*, as 0.1% aerosol or 0.05% cream, lotion, or ointment (potency expressed as betamethasone) applied 3 times a day as the aerosol or 2 times a day as other dosage forms to adults but only once a day to children.

Clocortolone Pivalate [9-Chloro-6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate [34097-16-0] $C_{27}H_{36}ClFO_5$ (495.03)]—Clocortolone pivalate is more than 10 times as potent as hydrocortisone when applied topically to the skin but has negligible systemic activity by this route. It has been used mainly in the treatment of eczema and atopic dermatitis but can probably be used to treat any glucocorticoid-responsive dermatosis or dermatitis. *Dose: Topical*, to the *skin*, *adults*, as 0.1% cream once or twice a day or by occlusive dressing.

Hydrocortisone Butyrate [Cortisol 17-butyrate; Cortisol Butyrate [13609-67-1]; $C_{25}H_{36}O_6$ (432.56)]—As a topical anti-inflammatory drug, hydrocortisone 17-butyrate is about 10 times as potent as hydrocortisone valerate but it is considerably less potent in causing systemic effects, such as adrenal suppression. It also appears to cause less cutaneous atrophy than other steroids described above. *Dose: Topical*, to the *skin*, as 0.1% ointment.

Hydrocortisone Valerate [11β,17,21-Trihydroxypregn-4-ene-3,20-dione 17-valerate [57524-89-7] $C_{26}H_{38}O_6$ (446.58); hydrocortisone 17-valerate; hydrocortisone valerate; Westcort (*Westwood*)]—Hydrocortisone valerate is used only for its topical anti-inflammatory properties. It is better absorbed than cortisol into the skin, from which site it is not transported as well into the blood. Consequently it has greater and longer-lasting local activity and lesser systemic activity and thus a greater therapeutic index than topical hydrocortisone. In the skin it is slowly converted to hydrocortisone; in the liver it is rapidly de-esterified and further metabolized. *Dose: Topical*, to the skin, as 0.2% cream 3 or 4 times a day to *adults* and once a day to *children*.

Desonide [11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone; 16-Hydroxyprednisolone 16,17-acetonide [638-94-8] $C_{24}H_{32}O_6$ (416.51); Tridesilon (*Miles*)]—A glucocorticoid of intermediate potency that is used only topically in the treatment of glucocorticoid-responsive skin diseases. It causes almost no systemic side effects; local side effects include burning sensations, itching, irritation, folliculitis, hypertrichosis, acneform eruptions, hypopigmentation, striae, cutaneous atrophy, milaria, and skin infections. Maceration from occlusive film may occur. *Dose: Topical*, to *skin*, thin film of 0.05% cream or ointment 2 to 3 times a day to *adults* and once a day to *children*.

Desoximetasone [9-Fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione [382-67-2] $C_{22}H_{29}FO_4$ (376.47); Topicort (*Hoechst-Roussel*)]—For syntheses see *CA 58:* 8388b, 1963; *Arzneimittel-Forschung 24:* 1, 1974. A white, crystalline powder; melts at about 217°. Insoluble in water; soluble in alcohol and chloroform; slightly soluble in water. A glucocorticoid of intermediate potency used only topically in the treatment of glucocorticoid-responsive dermatoses and dermatitides. As a 0.025% cream, it is approximately equieffective with 0.05% betamethasone dipropionate, 0.12% betamethasone valerate, and 0.1% triamcinolone. It is fluorinated and lacks a 17-OH group, so that systemic effects such as adrenal suppression can occur, and it must be used cautiously in infants and children. For adverse effects see the general statement in this section. *Dose: Topical*, to the *skin*, as 0.05 or 0.25% cream twice a day in adults and once a day in children.

Dexamethasone Acetate [Dexamethasone 21-acetate [1177-87-3] $C_{24}H_{31}FO_6$ (434.50); Decadron L.A. Suspension (*MSD*)]—The actions, uses, and side effects are those of dexamethasone (page 965). It is employed only as a repository form of dexamethasone, for systemic or intralesional use. *Dose* (dexamethasone equivalent): *Intramuscular, adults*, 8 to 16 mg every 1 to 3 weeks; *intralesional*, 0.8 to 1.6 mg/injection site; *intra-articular*, usually 4 to 16 mg every 1 to 3 weeks, if necessary.

Diflorasone Diacetate [6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-diacetate [33564-31-7] $C_{28}H_{32}F_2O_7$ (494.53); Florone (*Upjohn*); Maxiflor (*Herbert*)]—A topical glucocorticoid of high potency. From clinical trials, it has been claimed both that the 0.05% cream or ointment is equivalent to 0.05% fluocinonide cream and more effective than 0.1% hydrocortisone cream or 0.1% betamethasone valerate ointment. Because it is fluorinated it can be expected to have more systemic activity than hydrocortisone and hence should be used cautiously in children. *Dose: Topical*, to the *skin*, as 0.05% cream or ointment 2 to 4 times a day to *adults* and once a day to *children*.

Flunisolide [6α-Fluoro-11β,16α,17,21-tetrahydroxypregna-3,20-dione cyclic 16,17-acetal with acetone [3385-03-3] $C_{24}H_{31}FO_6$ (434.50); Syntaris (*Syntex*)]—For the synthesis refer to US Pat 3,124,571. *Uses:* A glucocorticoid used topically to treat *noninfectious rhinitis*. It has a half-life of 1 to 2 hr, which is about one-tenth that of beclomethasone, a steroid used for the same purpose. *Dose: Intranasal, adult, initially* 50 μg (2 metered sprays) into each nostril 2 times a day and, for *maintenance*, 25 μg (1 metered spray into each nostril once a day); *children 6 to 14 years* of age, initially 25 μg (one metered spray) into each nostril 3 times a day and, for *maintenance*, once a day. *Dosage Form:* Nasal spray: 25 μg/metered spray.

Fluprednisolone [6α-Fluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione [53-34-9] $C_{21}H_{27}FO_5$ (378.44); Alphadrol (*Upjohn*)]—Synthesis of fluprednisolone is described in RPS-15, page 898. A white to off-white, odorless, crystalline powder; melts at about 210°. Practically insoluble in water; sparingly soluble in alcohol; slightly soluble in chloroform, ether. A glucocorticoid with typical actions, uses, and side effects (see introduction in this section). It is approximately 2.5 times as potent as prednisolone and 40 times as potent as cortisone. However, occasionally it does not appear to be able to control allergic or inflammatory conditions that can be controlled with other glucocorticoids. It also has erratic mineralocorticoid activity. *Dose: Oral, adult*, initially 2.5 to 30 mg a day followed by 1.5 to 12 mg a day for maintenance. *Children*, for *maintenance*, 70 μg/kg (or 2.5 mg per m^2 of body surface) per day in 3 divided doses; for *disease*, 250 μg to 1 mg/kg (or 7.5 to 30 mg/m^2) a day in 3 or 4 divided doses. *Dosage Forms:* Tablets: 0.75 and 1.5 mg.

Halcinonide [21-Chloro-9-fluoro-11β,16α,17-trihydroxypregn-4-ene-3,20-dione cyclic 16,17-acetal with acetone [3093-35-4] $C_{24}H_{32}ClFO_5$ (454.97); Halog (*Squibb*)]—For synthesis see *J Org Chem 27:* 690, 1962. A white, crystalline powder; melts at about 265°, with decomposition. Insoluble in water; slightly soluble in alcohol. A high-potency glucocorticoid used for topical treatment of glucocorticoid-responsive dermatoses and dermatitides. Its clinical status remains to be determined. The systemic side effects of topical application are usually of a low degree, but care must be exercised in children. The cutaneous side effects are somewhat more severe than with drugs of lower potency (see page 960 for adverse effects and contraindications). *Dose: Topical*, to the *skin*, as 0.025 or 1% cream or 1% ointment or solution 2 or 3 times a day in adults and once a day in children.

Medrysone [11β-Hydroxy-6α-methylpregn-4-ene-3,20-dione [2668-66-8] $C_{22}H_{32}O_3$ (344.49); HMS Liquifilm (*Allergan*)]—A weak glucocorticoid effective in the treatment of allergic conjunctivitis and possibly in other mild superficial ocular inflammatory conditions. Untoward effects are stinging and burning sensations after instillation. *Dose: Topical*, to the eye, as 1% ophthalmic suspension. *Dosage Form:* Ophthalmic Suspension: 1%.

Methylprednisolone Sodium Phosphate [11β,17,20-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(disodium phosphate) [5015-36-1] $C_{22}H_{29}Na_2O_8P$ (498.42)]—The actions and uses of this water-soluble methylprednisolone derivative are essentially the same as those of methylprednisolone sodium succinate (page 969) except that the sodium phosphate is reported to yield about 20% higher plasma levels of methylprednisolone than the sodium succinate. *Dose:* See *Methylprednisolone Sodium Succinate*.

Prednisolone Acetate and Prednisolone Sodium Phosphate—When used in combination the soluble prednisolone sodium phosphate (see page 972) gives rise to rapidly achieved plasma or synovial fluid levels of prednisolone, whereas prednisolone acetate supposedly maintains the levels for a longer time because of its lower solubility. *Dose: Intramuscular, intra-articular*, or *intrasynovial*, in fixed ratio sterile suspension (1:4), 20 to 80 mg of prednisolone acetate and 5 to 20 mg of prednisolone sodium phosphate, repeated at 3 day- to 4-week intervals.

MOLECULAR BIOLOGY OF
THE CELL

SECOND EDITION

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into normal fibroblasts, the transfected cells are killed by the cytotoxic T cells described in the first experiment. These and other experiments suggest that fragments of viral proteins can both find their way to the cell surface and associate with class I MHC molecules.

It is not difficult to understand how viral proteins can be degraded in infected cells, since almost all cellular proteins are known to be continually degraded (see p. 418). It is more difficult to understand how fragments of the influenza nucleoprotein get to the cell surface, since the protein is synthesized on cytoplasmic ribosomes and would not normally have access to the lumen of the endoplasmic reticulum, where proteins destined for the cell surface usually begin their journey (see p. 412). T cell recognition probably requires very small amounts of antigen, however, so misrouting only a small fraction of the nucleoprotein fragments to the cell surface may create a target cell that an appropriate cytotoxic T lymphocyte can recognize.

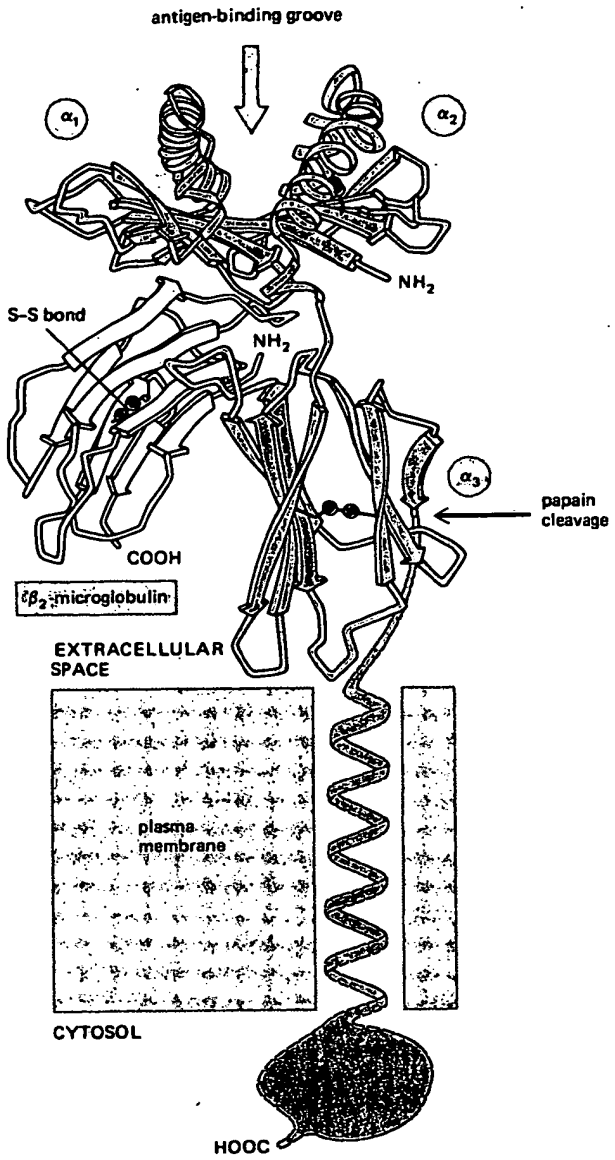
X-ray Diffraction Studies Show the Antigen-binding Site of a Class I MHC Glycoprotein[40]

A major advance in our understanding of how MHC molecules present antigen to T cells came in 1987, when the three-dimensional structure of a human class I MHC glycoprotein was obtained by x-ray crystallography. As shown in Figure 18–53A, the protein has a single putative antigen-binding site located at one end of the molecule. The site consists of a deep groove between two long α helices derived from the nearly identical α_1 and α_2 domains; the base of the groove is formed by eight β strands derived from the same two domains. The size of the groove is about 2.5 nm long, 10 nm wide, and 11 nm deep, which is large enough to accommodate a peptide of about 10 to 20 amino acid residues, depending on the extent to which the peptide is compressed by coiling or bending. Remarkably, the groove in the crystallized protein was not empty: it contained a small molecule of unknown origin, suspected to be a peptide, which co-purified and co-crystallized with the MHC glycoprotein (see Figure 18–53B). This finding strongly implicates the groove as the antigen-binding site and suggests that once a peptide binds to this site, it dissociates very slowly; this conclusion is supported by the observation that fibroblasts exposed for a short period to fragments of the influenza virus nucleoprotein remain targets for influenza-specific cytotoxic T cells for at least 3 days.

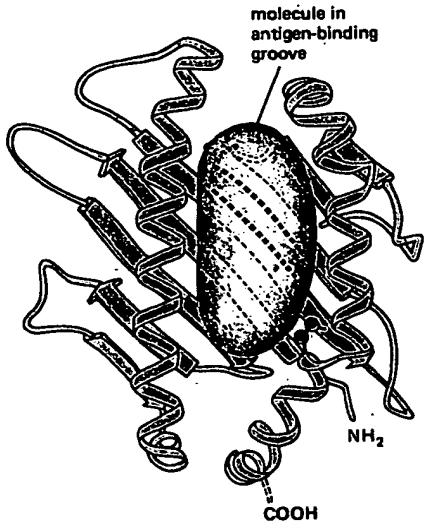
Most of the polymorphic amino acid residues in the MHC glycoprotein (those that vary between allelic forms of this type of molecule) are located inside the groove, where they would be expected to bind antigen, or on its edges, where they would be accessible for recognition by the T cell receptor. Presumably the variability in class I MHC molecules has been selected to allow them to bind and present many different virus-derived peptides. Nonetheless, it is still surprising that the small number of different antigen-binding sites associated with the class I MHC molecules in an individual (a maximum of six in humans) can bind the large number of virus-derived peptides that T cells can specifically recognize. Even more puzzling in this respect are the class II MHC glycoproteins, which are thought to have a three-dimensional structure very similar to that of class I molecules. Although an individual makes only about 10 to 20 types of class II molecules, each with its own unique antigen-binding site, these molecules seem to be able to bind and present an apparently unlimited variety of foreign peptides to *helper T cells*, which play a crucial part in almost all immune responses.

Helper T Cells Recognize Fragments of Foreign Antigens in Association with Class II MHC Glycoproteins on the Surface of Antigen-presenting Cells[41]

Helper T cells are required for most other types of lymphocytes to respond optimally to antigen. The crucial importance of helper T cells in immunity is dramatically demonstrated by the devastating epidemic of *acquired immunodeficiency syndrome (AIDS)*. The disease is caused by a retrovirus (human immuno-



(A) SIDE VIEW



(B) TOP VIEW

Figure 18–53 (A) The structure of a human class I MHC glycoprotein as determined by x-ray diffraction analysis of crystals of the extracellular part of the molecule. The extracellular part was cleaved from the transmembrane segment by the proteolytic enzyme papain. Each of the two domains closest to the plasma membrane (α_3 and β_2-microglobulin) resembles a typical immunoglobulin domain (see Figure 18–28B), while the two domains farthest from the membrane (α_1 and α_2) are very similar to each other and together form a groove at the top of the molecule that is believed to be the antigen-binding site. Class II MHC molecules are thought to have a very similar structure. (B) The putative antigen-binding groove viewed from above, containing the small molecule (thought to be a peptide) that co-purified with the MHC protein. This is also the part of the molecule that interacts with the T cell receptor. (After P.J. Bjorkman, M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley, *Nature* 329:506–512, 1987.)

deficiency virus, HIV) that kills helper T cells, thereby crippling the immune system and rendering the patient susceptible to infection by microorganisms that rarely infect normal individuals. As a result, most AIDS patients die of infection within several years of the onset of symptoms.

Before they can help other lymphocytes respond to antigen, helper T cells must first be activated themselves. This activation occurs when a helper T cell recognizes a foreign antigen bound to a class II MHC glycoprotein on the surface of a specialized **antigen-presenting cell.** Antigen-presenting cells are found in most tissues. They are derived from bone marrow and comprise a heterogeneous set of cells, including *dendritic cells* in lymphoid organs, *Langerhans cells* in skin, and certain types of macrophages. Together with B cells, which can also present antigen to helper T cells (see below), and thymus epithelial cells (see p. 1052), these specialized antigen-presenting cells are the main cell types that normally express class II MHC molecules (see Table 18–2).

Many kinds of experiments demonstrate the central importance of class II MHC molecules in presenting foreign antigens to helper T cells. The binding of antibodies to class II molecules on antigen-presenting cells, for example, blocks

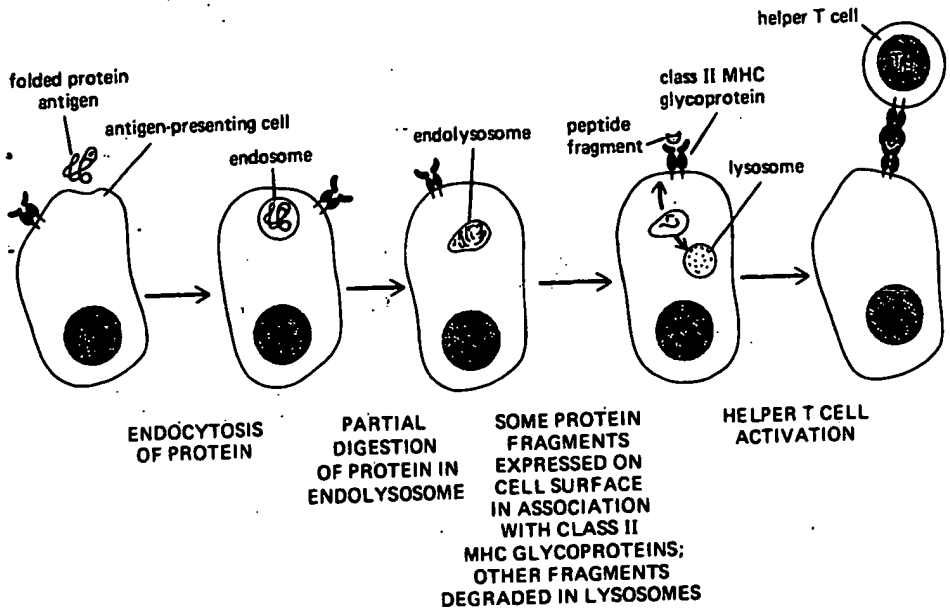


Figure 18–54 How protein antigens are thought to be "processed" and then displayed by an antigen-presenting cell. Since class II MHC glycoproteins have been found to recycle through the endosomal compartment, they may initially associate with peptide fragments in the endolysosomal compartment and then return to the cell surface with a bound peptide (not shown).

the ability of these cells to present foreign antigen to helper T cells. Moreover, fibroblasts, which do not make class II MHC molecules and cannot present foreign antigens to helper T cells, can be converted to effective antigen-presenting cells if they are transfected with a gene that codes for a class II MHC molecule.

Like the viral antigens presented to cytotoxic T cells, the antigens presented to helper T cells on antigen-presenting cells are usually degraded fragments of the foreign protein. These peptides are thought to be bound to class II MHC molecules in the same way that virus-derived peptides are bound to class I MHC molecules (see Figure 18–53). Unlike the virus-infected target of a cytotoxic T cell, however, the antigen-presenting cell does not synthesize the foreign protein. Instead, it is thought that the foreign protein is ingested by endocytosis and partially degraded in the acidic environment of endosomes or endolysosomes (see p. 331) before selected fragments are returned to the cell surface—a sequence of events collectively called **antigen processing** (Figure 18–54). Thus, if endocytosis is blocked by lightly fixing antigen-presenting cells with a chemical such as formaldehyde, or if proteolysis in endolysosomes and lysosomes is inhibited by a drug such as chloroquine, the cells are no longer able to process a foreign protein and present it to helper T cells. Cells treated in these ways, however, are still able to present the protein if it is cleaved into small peptides (10–15 amino acids long) before it is added to the cells.

A remarkable property of an antigen-presenting cell is that it can process and present virtually any antigen to an appropriate helper T cell. This lack of antigen specificity suggests that antigen-presenting cells take up antigen by fluid-phase rather than by receptor-mediated endocytosis (see p. 328). If this is so, then most of the proteins ingested and degraded will be host (self) proteins, whose peptide fragments will occupy the binding site of many of the class II MHC molecules. Presumably the binding of foreign peptides to only a small proportion of MHC molecules is sufficient to activate a helper T cell.

Helper T Cells Stimulate Activated T Lymphocytes to Proliferate by Secreting Interleukin-2[42]

Activation of a helper T cell is a complex process involving various secreted proteins called **interleukins**, which act as local chemical mediators. Activation is thought to begin when the T cell, by unknown means, stimulates the antigen-presenting cell to secrete one or more interleukins. The best characterized of these mediators is **interleukin-1 (IL-1)**. The combined action of IL-1 (and probably other interleukins) and antigen binding, however, do not stimulate helper T cell proliferation directly. Instead, they cause the T cell to stimulate its own proliferation by inducing it to secrete a growth factor called **interleukin-2 (IL-2)** as well as to

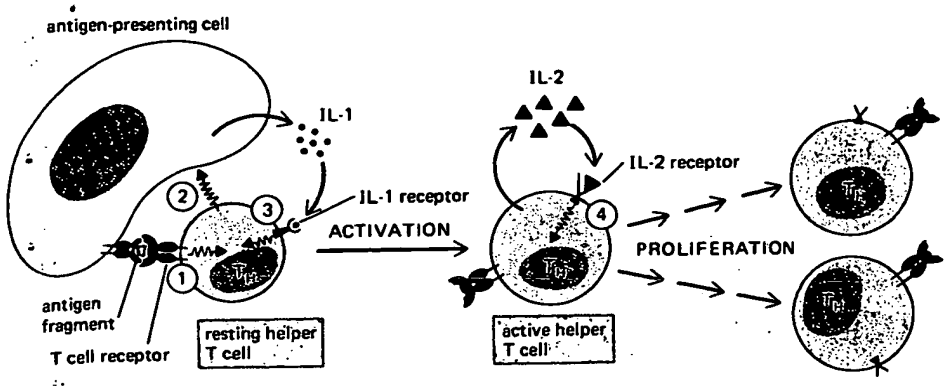


Figure 18–55 The sequence of signaling events believed to occur when antigen stimulates helper T cells to proliferate. Binding of the T cell to the antigen on the surface of an antigen-presenting cell signals the T cell receptor to trigger the inositol phospholipid cell-signaling pathway (see p. 702) (signal 1). This causes the T cell to stimulate the antigen-presenting cell by an unknown mechanism (signal 2). The antigen-presenting cell then secretes interleukins, such as interleukin-1 (IL-1), which help activate the T cell (signal 3). The activated T cell makes interleukin-2 (IL-2) receptors and secretes IL-2; the binding of IL-2 to its receptors (signal 4) stimulates the cell to grow and divide. When the antigen is eliminated, the T cells eventually stop producing IL-2 and IL-2 receptors, so cell proliferation stops.

synthesize cell-surface IL-2 receptors. It is the binding of IL-2 to these receptors that stimulates the T cell to proliferate. In this way the helper T cell can continue to proliferate, through an *autocrine mechanism* (see p. 690), after it has left the surface of the antigen-presenting cell (Figure 18–55). The helper T cell can also help stimulate the proliferation of any other T cells, including cytotoxic T cells, that have first been induced to express IL-2 receptors. Because the expression of IL-2 receptors is strictly dependent on antigen stimulation, however, this does not result in the indiscriminate proliferation of all T cells, but only those that have encountered antigen.

Once the requirements for T cell proliferation were discovered, it was possible to produce indefinitely proliferating, antigen-specific *T cell lines* in culture by continuously administering IL-2 and periodically stimulating the cells with antigen to maintain the expression of IL-2 receptors. Single cells from such lines could then be isolated to generate **T cell clones.** As we have seen, such clones have been critically important in T cell research. They made it possible, for example, to isolate T cell receptors and their genes; they have also been widely used to study the mechanisms of T cell activation and the role of helper T cells in stimulating the responses of other lymphocytes.

Helper T Cells Are Required for Most B Cells to Respond to Antigen[43]

Helper T cells are essential for B cell antibody responses to most antigens. This was first discovered in the mid-1960s through experiments in which either thymus cells or bone marrow cells were injected together with antigen into irradiated mice. Mice that had received only bone marrow or only thymus cells were unable to make antibody; but if a mixture of thymus and bone marrow cells was injected, large amounts of antibody were produced. It was later shown that the thymus provides T cells, while the bone marrow provides B cells (Figure 18–56). The use of a specific chromosome marker to distinguish between the injected T and B cells showed that the antibody-secreting cells are B cells, leading to the conclusion that T cells must help B cells respond to antigen.

There are some antigens, however, including many microbial polysaccharides, that can stimulate B lymphocytes to proliferate and mature without T cell help. Such *T-cell-independent antigens* are usually large polymers with repeating, identical antigenic determinants whose multipoint binding to the membrane-bound antibody molecules that serve as antigen receptors on B cells may generate a strong enough signal to activate B cells directly. There is evidence that the cells that respond to multimeric antigens in this way are mainly a separate subset of B cells that has evolved to react against microbial polysaccharides without T-cell help.

Helper T Cells Help Activate B Cells by Secreting Interleukins[44]

Once activated by foreign antigen on the surface of a specialized antigen-presenting cell, an appropriate helper T cell can help activate a B cell by binding to the same foreign antigen on the B cell surface. The antigen-presenting cell ingests and presents antigens nonspecifically (see p. 1046), but a B cell generally

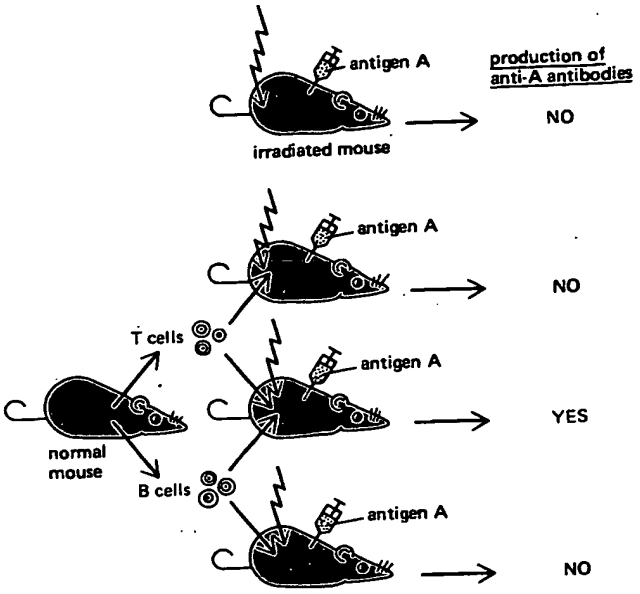


Figure 18–56 The experiment that first suggested that both T cells and B cells are required if an animal is to make antibody responses. The dose of irradiation used kills the T cells and B cells of the irradiated mouse.

presents only an antigen that it specifically recognizes. The antigen is selected by its binding to the specific membrane-bound antibodies (antigen receptors) on the surface of the B cell; it is ingested by receptor-mediated endocytosis (see p. 328) and is then degraded and recycled to the cell surface in the form of peptides bound to class II MHC glycoproteins for recognition by the helper T cell. Thus the helper T cell recognizes the same antigen-MHC complexes on the B cell it helps as on the antigen-presenting cell that initially activated the T cell.

The specific contact between a helper T cell and a B cell initiates an internal rearrangement of the helper cell cytoplasm that orients the centrosome and Golgi apparatus toward the B cell, as described previously for a cytotoxic T cell contacting a target cell (see Figure 18–47). In this case, however, the orientation is thought to enable the helper T cell to direct the secretion of interleukins (and perhaps to focus membrane-bound signaling molecules) onto the B cell surface. These interleukins include *IL-4*, which helps initiate B cell activation, *IL-5*, which stimulates activated B cells to proliferate, and *IL-6*, which induces activated B cells to mature into antibody-secreting cells. Some of these and other interleukins can induce B cells to switch from making one class of antibody to making another (see p. 1029). Some of the signals thought to be involved in the initial activation of a B cell are illustrated in Figure 18–57.

How do signals pass from activated cell-surface receptors to the cell interior when B or T cells are stimulated by antigen and interleukins? The answer is not known for interleukin receptors, but there is strong evidence that receptors for antigen on both B and T cells signal the cell by activating the inositol phospholipid pathway discussed in Chapter 12 (see p. 702).

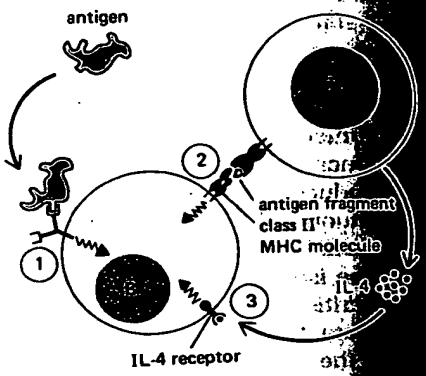


Figure 18–57 At least three types of signals are likely to be involved in the initial stages of B cell activation. The relative importance of these signals is uncertain and may vary depending on the type of B cell and antigen. Signal 1 is caused by antigen binding and is thought to be mediated by the inositol phospholipid cell-signaling pathway (see p. 702); it helps activate the B cell and may induce the expression of receptors for some of the helper-T-cell-derived interleukins. The B cell then ingests and degrades the antigen (not shown) and presents small fragments of the antigen to the helper T cell in association with class II MHC molecules. It is not clear if T cell binding signals the B cell (shown here as signal 2) or only serves to focus the secretion of interleukin-4 (IL-4) and other interleukins (not shown) onto the B cell surface (signal 3). In addition to activating the B cell, signal 3 stimulates the cell to make more class II MHC glycoprotein, thereby increasing the ability of the B cell to receive T cell help. Once the B cell is activated, other helper-T-cell-derived interleukins (such as IL-5, IL-6, and γ-interferon) help induce the cell to proliferate and mature into an antibody-secreting cell (not shown).

Some Helper T Cells Activate Macrophages by Secreting γ-Interferon[45]

Helper T cells do not confine their help to lymphocytes. Those helper T cells that secrete IL-2 when stimulated by antigen also secrete other interleukins, such as *γ-interferon*, that attract macrophages and activate them to become more efficient at phagocytosing and destroying invading microorganisms. The ability of T cells to attract and activate macrophages is especially important in defense against infections by microorganisms that can survive simple phagocytosis by nonactivated macrophages. Tuberculosis is one such infection.

The antigen-triggered secretion of γ-interferon and other macrophage-activating interleukins by helper T cells underlies the familiar tuberculin skin test. If tuberculin (an extract of the bacterium responsible for tuberculosis) is injected

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CHAPTER

63 ADRENOCORTICOTROPIC HORMONE; ADRENOCORTICAL STEROIDS AND THEIR SYNTHETIC ANALOGS; INHIBITORS OF ADRENOCORTICAL STEROID BIOSYNTHESIS

Robert C. Haynes, Jr., and Ferid Murad

Adrenocorticotropic hormone (ACTH, corticotropin) and the steroids of the adrenal cortex are considered together in this chapter because the primary physiological and pharmacological effects of ACTH result from the secretion of adrenocortical steroids. Biologically active synthetic analogs of the adrenocorticosteroids are also included, as are substances that alter the pattern of secretion of the adrenal cortex by inhibiting certain biosynthetic reactions. Synthetic steroids and other compounds that inhibit the action of aldosterone on the renal tubule are discussed in Chapter 36.

History. The physiological significance of the adrenals began to be appreciated as a consequence of the description by Addison (1855) of the clinical syndrome resulting from destructive disease of the adrenal glands. His observations interested the physiologist Brown-Séquard (1856), who did the pioneer experiments on the effects of adrenalectomy and concluded that the adrenal glands are essential to life.

By the third decade of this century it was generally recognized that the cortex rather than the medulla is the life-maintaining portion of the gland. Soon the literature was replete with descriptions of the numerous physiological abnormalities exhibited by adrenalectomized animals. The complex nature of adrenocortical deficiency was dramatized in the 1930s by the partisan character of research groups oriented to study either the imbalance of electrolytes or the defects in carbohydrate metabolism present in the deficient state. Renal loss of sodium was convincingly demonstrated to be a characteristic of adrenocortical insufficiency by Harrop and associates (1933) as well as by Loeb and coworkers (1933). Equally convincing was the demonstration of a depletion of carbohydrate stores (Cori and Cori, 1927). Furthermore, hypoglycemia could be corrected by adrenocortical extracts (Britton and Silvette, 1931). Glucose and glycogen, formed under the influence of the adrenal cortex during fasting, appeared to be derived from tissue protein (Long *et al.*, 1940). From these studies there emerged the concepts of two types of adrenocortical hormones. The mineralocorticoids primarily regulate electrolyte homeostasis, and the glucocorticoids are hormones concerned with carbohydrate metabolism. This concept of the dichotomy of "salt" and "sugar" hormones (mineralocorticoids and glucocorticoids) has proven useful and survives at the present time in a modified form.

In 1932, the neurosurgeon Cushing described the syndrome of hypercorticism, which bears his name (Cushing, 1932). The cases Cushing described were those of "pituitary basophilism," recognized subsequently as being a condition characterized by hypersecretion of ACTH. The symptom complex is now known to result from excessive plasma concentrations of adrenocortical hormones, regardless of whether they originate endogenously or as the consequence of therapeutic intervention.

The preparation of adrenocortical extracts with a reasonable degree of activity was first accomplished in 1930 by Swingle and Pfiffner and by Hartman and associates. The existence of biologically active tissue extracts presented a challenge to organic chemists, who by 1942 had isolated, crystallized, and elucidated the structures of 28 steroids from the adrenal cortex (Reichstein and Shoppee, 1943). Five of these compounds—cortisol (hydrocortisone), cortisone, corticosterone, 11-dehydrocorticosterone, and 11-desoxycorticosterone—were demonstrated to be biologically active. Another decade passed before the principal mineralocorticoid was discovered. Deming and Luetscher (1950) found that extracts of urine from patients with edema induced sodium retention and potassium excretion in adrenalectomized rats. The definitive evidence for the source of the active material was provided by Tait and coworkers (1952), who purified the compound with this activity from adrenocortical extracts. The substance was crystallized, the structure was established, and the hormone was eventually named *aldosterone* (Simpson *et al.*, 1954).

Meanwhile, other investigators had turned their attention to the adenohypophysis. The classical studies of Foster and Smith (1926) established the fact that hypophysectomy results in atrophy of the adrenal cortex. By 1933, it had been demonstrated

that cell-free extracts of the anterior pituitary had a stimulating effect upon the adrenal cortex of the hypophysectomized animal. Further chemical fractionation of such extracts led to the isolation of a hormone, ACTH, that acted selectively to cause chemical and morphological changes in the adrenal cortex (Li *et al.*, 1943; Sayers *et al.*, 1943; Astwood *et al.*, 1952). The structure of ACTH was established by Bell and coworkers (1956). Within a few years biologically active peptides were synthesized (Hofmann *et al.*, 1961), as was an ACTH of 39 amino acid residues (Schwyzer and Sieber, 1963). The rate of release of ACTH from the adenohypophysis was shown to be determined by the balance of inhibitory effects of the hormones of the adrenal cortex (Ingle *et al.*, 1938) and the excitatory effects of the nervous system. The hypothalamus was established as the "final common path" for the variety of stimuli impinging on the adenohypophysis.

A detailed analysis of the morphology of the adrenal cortex had suggested to Swann (1940) and to Deane and Greep (1946) that the zona glomerulosa of the adrenal cortex functions relatively independently of the pituitary. Following hypophysectomy, the zona glomerulosa thickens, whereas the fasciculata shrinks markedly and the reticularis disappears almost entirely. These morphological observations, together with the fact that the hypophysectomized rat, in contrast to the adrenalectomized animal, can survive without salt therapy, prompted Swann as well as Deane and Greep to assign to the zona glomerulosa the specific function of autonomously elaborating a hormone regulating electrolyte balance. This hormone is now known to be aldosterone. Subsequent experimental studies have shown that the rate of secretion of aldosterone is regulated by a complex system, of which the pituitary is but one element.

In 1949, Hench and coworkers announced the dramatic effects of cortisone and ACTH in the treatment of rheumatoid arthritis. As early as 1929, Hench was impressed by the fact that arthritic patients, when pregnant or jaundiced, experienced a temporary remission; he believed that a metabolite was responsible for the remission. The possibility that the antirheumatic substance might be an adrenocortical hormone was entertained, and as soon as cortisone was available in sufficient quantity it was tested in a case of acute rheumatoid arthritis. Fortunately, an adequate dose was employed and the response was dramatic. Thereafter, the salutary effects of ACTH were also demonstrated. The observations (Hench *et al.*, 1949) immediately evoked wide interest. Soon, therapeutic applications were extended to other diseases, with results to be presented later in this chapter. The impact upon the medical world can be appreciated from the fact that, in the year following the first published report of the efficacy of cortisone in the treatment of rheumatoid arthritis, the Nobel Prize in Medicine was jointly awarded to Kendall and Reichstein, who were responsible for much of the basic chemical research that led to the synthesis of the steroid, and to Hench, whose contribution has just been described.

In addition to a surge of clinical investigation, the therapeutic success of cortisone stimulated a wave of basic research in the 1950s. In that decade knowledge of the biochemistry of adrenal steroid synthesis and metabolism was brought close to its present level. As noted above, aldosterone was discovered; it was established that ACTH controls the reaction of cholesterol side chain scission (Stone and Hechter, 1954) and acts through the intermediacy of adenosine 3',5'-monophosphate (cyclic AMP) (Haynes *et al.*, 1959); most synthetic analogs of cortisol used today were introduced, and practical technics for determination of cortisol became available to the clinician.

Effective clinical use of the corticosteroids has become possible because of their isolation, elucidation of structure, and economical synthesis. Manipulation of structure has yielded a variety of synthetic analogs, some of which represent significant therapeutic gains in terms of the ratio of anti-inflammatory potency to effects on electrolyte metabolism. However, hopes for elimination of toxicity have not been fulfilled. For this reason, it cannot be overemphasized that the corticosteroids, in pharmacological doses, are powerful drugs with slow cumulative toxic effects on many tissues which may not be apparent until made manifest by a catastrophe.

ADRENOCORTICOTROPIC HORMONE

Chemistry. The structure of human ACTH, a peptide of 39 amino acid residues, is shown in Figure 63–1. Loss of one amino acid from the N-terminal end of the molecule by hydrolytic cleavage results in complete loss of biological activity. In contrast, a number of amino acids may be split off the C-terminal end with no effect on potency. A 20-amino acid peptide (sequence 1 through 20, Figure 63–1) retains the activity of the parent hor-

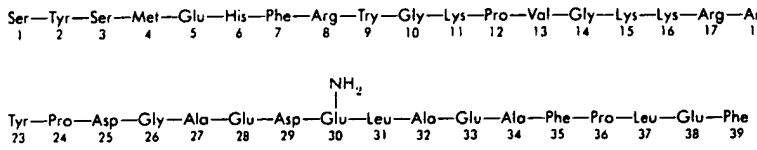


Figure 63–1. *Amino acid sequence of human ACTH.*

Ovine, porcine, and bovine ACTHs differ from human ACTH only at amino acid positions 25, 31, and 33 (Li, 1972).

mone. The structure-activity relationship of ACTH has been reviewed by Otsuka and Inouye (1975). The structural relationships between ACTH, endorphins, lipotropins, and the melanocyte-stimulating hormones are discussed in Chapter 59.

Actions on Adrenal Cortex. ACTH stimulates the human adrenal cortex to secrete cortisol, corticosterone, aldosterone, and a number of weakly androgenic substances. In the absence of the adenohypophysis, the adrenal cortex undergoes atrophy and the rates of secretion of cortisol and corticosterone, which are markedly reduced, do not respond to otherwise-effective stimuli. Although ACTH can stimulate secretion of aldosterone, the rate of secretion is relatively independent of the adenohypophysis, and this explains the nearly normal electrolyte balance in the hypophysectomized animal. The zona glomerulosa is the least affected by atrophic changes that follow hypophysectomy, and it is the glomerulosa that is mainly responsible for the elaboration of aldosterone.

Prolonged administration of large doses of ACTH induces hyperplasia and hypertrophy of the adrenal cortex with continuous high output of cortisol, corticosterone, and androgens.

Mechanism of Action. ACTH acts to stimulate the *synthesis* of adrenocortical hormones; if it facilitates the release of preformed steroids from the adrenal cortex at all, this effect is overshadowed by the greater effect on synthesis. ACTH, as many other hormones, controls its target tissue through the agency of cyclic AMP. Thus, treatment with ACTH causes an increase in concentration of the cyclic nucleotide within adrenocortical cells (Haynes, 1958); cyclic AMP mimics ACTH in stimulating steroidogenesis (Haynes *et al.*, 1959) and in maintaining the weight of the adrenal after hypophysectomy (Ney, 1969). ACTH reacts with a specific hormone receptor in the adrenal-cell plasma membrane, and the result is a stimulation of adenylate cyclase activity and the formation of cyclic AMP.

The principal metabolic site at which steroidogenesis is regulated by the cyclic nucleotide is the oxidative cleavage of the side chain of cholesterol, the reaction that results in the formation of pregnenolone (*see* Figure 63–3, page 1465). This step is rate limiting in the sequence of reactions that leads to the formation of adrenal steroid hormones (Stone and Hechter, 1954). Exposure of adrenocortical cells to ACTH together with aminoglutethimide (to block side chain cleavage; *see* below) leads to increased amounts of cholesterol within the adrenal mitochondria, the locus of the side chain–cleaving enzyme (Mahaffee *et al.*, 1974). Furthermore, cholesterol bound to cytochrome P-450 is increased by ACTH (Bell and Harding, 1974; Paul *et al.*, 1976). These findings, together with evidence that the availability of cholesterol is the factor that limits the rate of the cleavage reaction in intact mitochondria (Kahnt *et al.*, 1974), suggest that ACTH, via cyclic AMP, stimulates the initial reaction in steroidogenesis from cholesterol by making the substrate available in increased concentration to the enzyme within the mitochondria. ACTH stimulates the formation of free cholesterol in the gland by activating cholesterol esterase, and this activation is apparently accomplished by phosphorylation of the enzyme (Beckett and Boyd, 1975; Pittman and Steinberg, 1977). ACTH also acts to increase the availability of cholesterol by stimulating its uptake from plasma lipoproteins (Gwynne *et al.*, 1976).

The trophic effects of ACTH on the adrenal cortex are little understood beyond the fact that they, like stimulation of steroidogenesis, appear to be mediated by cyclic AMP (Ney, 1969). The regulation of the adrenal cortex by ACTH has been reviewed by Kimura (1981).

Extra-adrenal Effects of ACTH. Large doses of ACTH given to adrenalectomized animals cause a number of metabolic changes, including ketosis, lipolysis, hypoglycemia (early after administration), and resistance to insulin (late after administration). These extra-adrenal effects are of doubtful physiological significance, particularly since large doses are needed to induce them (Engel, 1961). Intravenous administration of ACTH (synthetic or porcine, but not bovine) leads to a transient elevation of the concentration of growth hormone in the plasma of adults but not children (Lee *et al.*, 1973).

Natural and synthetic corticotropins darken the isolated skin of the frog; this is not surprising since the amino acid sequence, 1 through 13, is identical with that of the melanocyte-stimulating hormone, α-MSH. Large doses of highly purified α-MSH and ACTH have been demonstrated to darken the skin of adrenalectomized human subjects. The hyperpigmentation of the skin that occurs in Addison's disease is thought to result from the high concentrations of ACTH that circulate in this condition (Thody, 1977; *see* Chapter 59).

Regulation of the Secretion of ACTH.

The fluctuations in the rates of secretion of cortisol, corticosterone, and, to some extent, aldosterone are determined by the fluctuations in the release of ACTH from the adenohypophysis. The adenohypophysis, in turn, is under the influence of the *nervous system* and *negative-feedback control* exerted by *corticosteroids* (*see* Gann *et al.*, 1981).

Nervous System: The Final Common Path. Stimuli that induce release of ACTH travel by neural paths converging on the median eminence of the

hypothalamus. The functional link between the median eminence and the adenohypophysis, the final common path, is vascular, not neural. In response to an appropriate stimulus, corticotropin-releasing factor (CRF) is elaborated at neuronal endings in the median eminence and transported in the hypophyseal-portal vessels to the adenohypophysis, where it stimulates the secretion of ACTH. The isolation and synthesis of an ovine CRF were reported by Vale and coworkers (1981). This polypeptide, which contains 41 amino acid residues, increases the concentrations of ACTH and cortisol in plasma when given intravenously to man. It does not change the concentrations of prolactin, growth hormone, thyrotropin, or the gonadotropins (Grossman *et al.*, 1982). Intravenous injection of 100 μg of CRF causes an exaggerated response in patients with Cushing's syndrome due to pituitary hyperfunction. It is thus useful in determining the cause of the disease and helps to rule out ectopic production of ACTH or functional tumors of the adrenal cortex as responsible (Muller *et al.*, 1983). (For additional information, *see* Conference, 1985.)

ACTH is synthesized in basophilic cells of the adenohypophysis and, like many other peptide hormones, it is derived from a larger precursor; the prohormone is a glycoprotein of about 30,000 molecular weight. As indicated in Figure 59–2 (page 1380), the precursor of ACTH includes the sequences of MSH, the lipotropins, and the endorphins. In man, the role of these three groups of active peptides remains conjectural and a subject of active investigation. The complex processing of the prohormone to ACTH, β-lipotropin, and other peptides has been studied extensively (*see* Loh and Loriaux, 1982).

Negative Feedback of the Corticosteroids (Cortisol and Corticosterone). Administration of certain corticosteroids suppresses the secretion of ACTH, reduces the store of ACTH in the adenohypophysis, and induces morphological changes (hyalinization of the basophilic cells) suggestive of functional impairment of the adenohypophysis. The adrenal cortex itself undergoes atrophy. In contrast, adrenalectomized animals and patients with Addison's disease have abnormally high concentrations of ACTH in the blood even under optimal environmental conditions. When a stimulus is applied to an adrenalectomized animal, the concentration of ACTH reaches even higher levels. These observations point out the important inhibitory role of the corticosteroids and clearly demonstrate that ACTH release remains under control of the nervous system in the absence of corticosteroid feedback. Secretion of ACTH at any instant is determined by the balance of neural excitatory and corticosteroid inhibitory effects.

Mechanism of Feedback by Corticosteroids. Binding of glucocorticoids has been detected in the pituitary, hypothalamus, and other areas of the brain (McEwen, 1979); however, the link between such binding and inhibition of secretion of ACTH has not been established. There is evidence of control at both hypothalamic and hypophyseal sites (*see* Gann *et al.*, 1981). Nakanishi and coworkers (1977) demonstrated that glucocorticoids cause a decrease in the level of mRNA for ACTH in the pituitary, suggesting control may be at least in part at the transcriptional level. It should be noted, however, that glucocorticoids can cause a fall in the plasma concentration of ACTH that is so rapid that other mechanisms may also be utilized (Watson *et al.*, 1979).

Examples of Effective Stimuli of Secretion. A number of conditions have been demonstrated to stimulate adrenocortical secretion in man. These include the agonal state, severe infections, shock, parturition, cold, exercise, and emotional stress. Stressful stimuli override the normal negative feedback control mechanisms, and plasma concentrations of adrenocortical steroids can be elevated within a few minutes of the initiation of an appropriate stimulus.

Diurnal Cycles in Adrenocortical Activity. The rate of secretion of cortisol by the adrenal cortex of a normal human subject under optimal conditions is about 20 mg per day. However, the rate is not steady and exhibits rhythmic fluctuations; concentrations of adrenocortical steroids in plasma are relatively high in the early-morning hours, decline during the day, and reach a minimum about midnight. Plasma concentrations of ACTH are higher at 6 A.M. than at 6 P.M. The diurnal patterns of adrenocorticoids and ACTH are not observed in patients with Cushing's disease, and this fact is considered in the diagnosis of the disorder.

Absorption and Fate. ACTH is readily absorbed from parenteral sites, and it is usually administered by intramuscular injection and occasionally by intravenous infusion. The hormone rapidly disappears from the circulation following its intravenous administration; in man, the half-life in plasma is about 15 minutes because of rapid enzymatic hydrolysis.

Bioassay. The USP has adopted the *Third International Standard for Corticotropin* (Bassett *et al.*, 1962) as the reference standard in the United States. Potency is based on an assay in hypophysectomized rats in which depletion of adrenal ascorbic acid is measured after subcutaneous administration of the ACTH. All commercial preparations are now described in these units only.

Preparations, Dosage, and Routes of Administration. *Corticotropin for injection* (ACTH) is available as a lyophilized powder (ACTHAR) for

cutaneous, intramuscular, or intravenous use. The preparation is derived from the pituitaries of mammals used for food. Maximal adrenocortical secretion is obtained in adults with a total dose of 25 USP units infused intravenously for 8 hours.

Repository corticotropin injection (CORTROPHIN GEL, H.P. ACTHAR GEL) is administered either intramuscularly or subcutaneously. It is a highly purified ACTH in gelatin solution. Typical doses are 40 to 80 units, given every 1 to 3 days. *Corticotropin zinc hydroxide suspension* (CORTROPHIN-ZINC) is a preparation of purified corticotropin adsorbed on zinc hydroxide, intended for intramuscular injection. Again, usual doses are 40 to 80 units every 1 to 3 days.

Cosyntropin (CORTROSYN) is a synthetic peptide corresponding to amino acid residues 1 to 24 of human ACTH. This preparation, approved for diagnostic purposes, is given intramuscularly or intravenously in a dose of 0.25 mg (equivalent to 25 units).

Therapeutic and Diagnostic Applications of ACTH. At the present time, the most important use of ACTH is as a *diagnostic agent* in adrenal insufficiency. For this purpose, ACTH is administered and the concentration of cortisol in plasma is determined. A normal increase in plasma cortisol rules out primary adrenocortical failure. If there is no acute response, prolonged or repeated administration of ACTH may be required. In cases of pituitary insufficiency, prolonged treatment can be expected to elicit a rise in plasma cortisol concentration.

Therapeutic uses of ACTH have included the treatment of adrenocortical insufficiency and nonendocrine disorders that are responsive to glucocorticoids. However, therapy with ACTH is less predictable and much less convenient than is that with appropriate steroids. Furthermore, ACTH stimulates secretion of mineralocorticoids and, therefore, may cause acute retention of salt and water. While this generally does not persist with continuing therapy, it is a potentially serious problem in patients who have cardiac insufficiency. ACTH would obviously be of no value in the treatment of primary adrenocortical failure. Furthermore, there is no substantial evidence that therapeutic goals can be attained with ACTH in secondary adrenocortical insufficiency that cannot be attained with appropriate doses of currently available steroids. It must be kept in mind, however, that ACTH and corticosteroids are not pharmacologically equivalent. Treatment with ACTH exposes the tissues to a mixture of glucocorticoids, mineralocorticoids, and androgens, in contrast to the conventional, contemporary practice of administering a single glucocorticoid. It is possible that the steroid mixture resulting from adrenal stimulation by ACTH has effects that differ significantly from those of a single, synthetic glucocorticoid. Thus, Grahame (1969) reported the absence of dermal atrophy in patients treated for prolonged periods of time with ACTH, in contrast to that found with corticosteroid treatment. This has been tentatively attributed to a protective action of androgens against the inhibitory effects of glucocorticoids on fibroblasts (Harvey and Grahame, 1973).

Clinical Toxicity of ACTH. The toxicity of ACTH, aside from rare hypersensitivity reactions, is entirely attributable to the increased rate of secretion of adrenocorticosteroids (*see* below). Hypersensitivity reactions, ranging from mild fever to anaphylaxis and death, have been reported. The synthetic ACTH peptides are thought to be less antigenic than is the parent molecule. Nevertheless, hypersensitivity to them does occur (Forssman and Mulder, 1973). Because it stimulates synthesis and secretion of mineralocorticoids and androgens, ACTH causes more sodium retention, a greater degree of hypokalemic alkalosis, and more acne than do the synthetic congeners of cortisol.

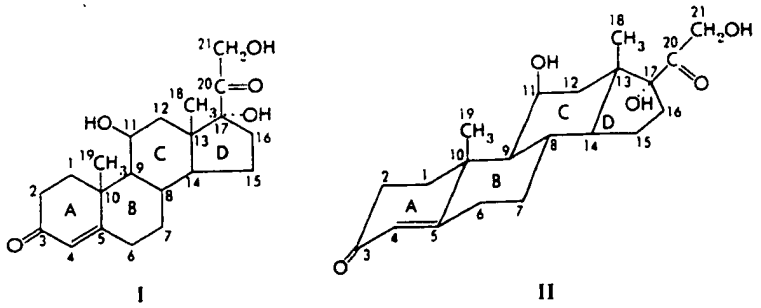
ADRENOCORTICAL STEROIDS

The adrenal cortex synthesizes two classes of steroids: the corticosteroids (glucocorticoids and mineralocorticoids) with 21 carbon atoms and the androgens with 19. A typical corticosteroid, *cortisol*, is shown in Figure 63–2; typical androgens are shown in Figure 63–3.

Adrenocorticosteroid Biosynthesis. Cholesterol is an obligatory intermediate in the biosynthesis of corticosteroids. Although the adrenal cortex synthesizes cholesterol from acetate by processes similar to those in liver, the greater part of the cholesterol (60 to 80%) utilized for corticosteroidogenesis comes from exogenous sources, both at rest and following administration of ACTH. Adrenocortical cells thus have large numbers of receptors that mediate the uptake of low-density lipoprotein, the predominant source of cholesterol (*see* Chapter 34). Cholesterol is enzymatically converted to 21-carbon corticosteroids and 19-carbon weak androgens by a series of steps presented in simplified form in Figure 63–3. Most of the reactions are catalyzed by mixed-function oxidases that contain cytochrome P-450 and require NADPH and molecular oxygen.

In addition to other androgens, the adrenal cortex secretes testosterone; however, about half the plasma testosterone of normal women is derived from androstenedione at an extra-adrenal site.

Adrenocorticosteroids are not stored in the adrenal. The amounts of corticosteroids found in adrenal tissue are insufficient to maintain normal rates of secretion for more than a few minutes in the absence of continuing biosynthesis. For this reason, the rate of biosynthesis is tantamount to the rate of secretion. Table 63–1 shows typical rates of secretion of the physiologically most important corticosteroids in man—cortisol and aldosterone—and also their approximate concentrations in peripheral plasma. The mechanism of control of steroidogenesis by ACTH has been discussed above, and the regulation of aldosterone synthesis by renin and angiotensin is described in Chapter 27.



I II

Figure 63–2. *Structure, stereochemistry, and nomenclature of adrenocorticosteroids, as typified by cortisol (hydrocortisone).*

The four rings—A, B, C, and D—are not in a flat plane, as conventionally represented in *I*, but have the approximate configuration shown in *II*. (The planarity of the valence angles about the double bond between C 4 and C 5 prevents the chair form of ring A, as shown, from being an energetically probable conformational state. As a result, ring A is in a half-chair conformation, not easily represented in two dimensions.) Orientation of the groups attached to the steroid ring system is importantly related to biological activity. The methyl groups at C 18 and C 19, the hydroxyl group at C 11, and the two-carbon ketol side chain at C 17 project above the plane of the steroid and are designated β. Their connection to the ring system is shown by full-line bonds. The hydroxy at C 17 projects below the plane and is designated α, and the connection to the ring is shown by a dotted bond. The ketone at C 3 in association with the double bond between C 4 and C 5 in ring A is an important structural feature of the biologically active corticosteroids. Reduction of the ketone at C 3 leads to the formation of two isomers: one, 3β-hydroxy; the other, 3α-hydroxy. Saturation of the 4,5 double bond leads to the formation of two isomers: 5α and 5β. Reduction of the ketone at C 20 creates an asymmetrical carbon at this site, the two possible isomers being designated α and β.

In formal chemical nomenclature, the adrenocortical hormones are described as derivatives of androstane or of pregnane. Double bonds are indicated by the symbol Δ with superscripts to indicate the position of the double bond. In this convention, cortisol is designated 11β,-17α,21-trihydroxy-Δ^4-pregnene-3,20-dione. Dehydroepiandrosterone is designated 3β-hydroxy-Δ^5-androsten-17-one.

PHYSIOLOGICAL FUNCTIONS AND PHARMACOLOGICAL EFFECTS

The effects of the corticosteroids are numerous and widespread. They influence carbohydrate, protein, and lipid metabolism; electrolyte and water balance; and the functions of the cardiovascular system, the kidney, skeletal muscle, the nervous system, and other organs and tissues. Furthermore, the corticosteroids endow the organism with the capacity to resist many types of noxious stimuli and environmental change. The adrenal cortex is the organ *par excellence*, of homeostasis, being responsible to a large extent for the relative freedom that higher organisms exhibit in a constantly changing environment. In the absence of the adrenal cortex, survival is possible but only under the most rigidly prescribed conditions; for example, food must be available regularly, sodium chloride ingested in relatively large quantities, and environmental temperature maintained within a suitably narrow range.

A given dose of corticosteroid may be *physiological* or *pharmacological*, depending on the environment and the activities of the organism. Under favorable conditions a small dose of corticosteroid maintains the adrenalectomized animal in a state of well-being. Under adverse conditions a relatively large dose is needed if the animal is to survive. This same large dose given repetitively under optimal conditions induces

Table 63–1. RATES OF SECRETION AND TYPICAL PLASMA CONCENTRATIONS OF THE MAJOR BIOLOGICALLY ACTIVE CORTICOSTEROIDS IN MAN

		CORTISOL	ALDOSTERONE
Rate of secretion under optimal conditions, mg/day		20	0.125
Concentrations in peripheral plasma of man, μg/dl	8 A.M.	16	0.01
	4 P.M.	4	

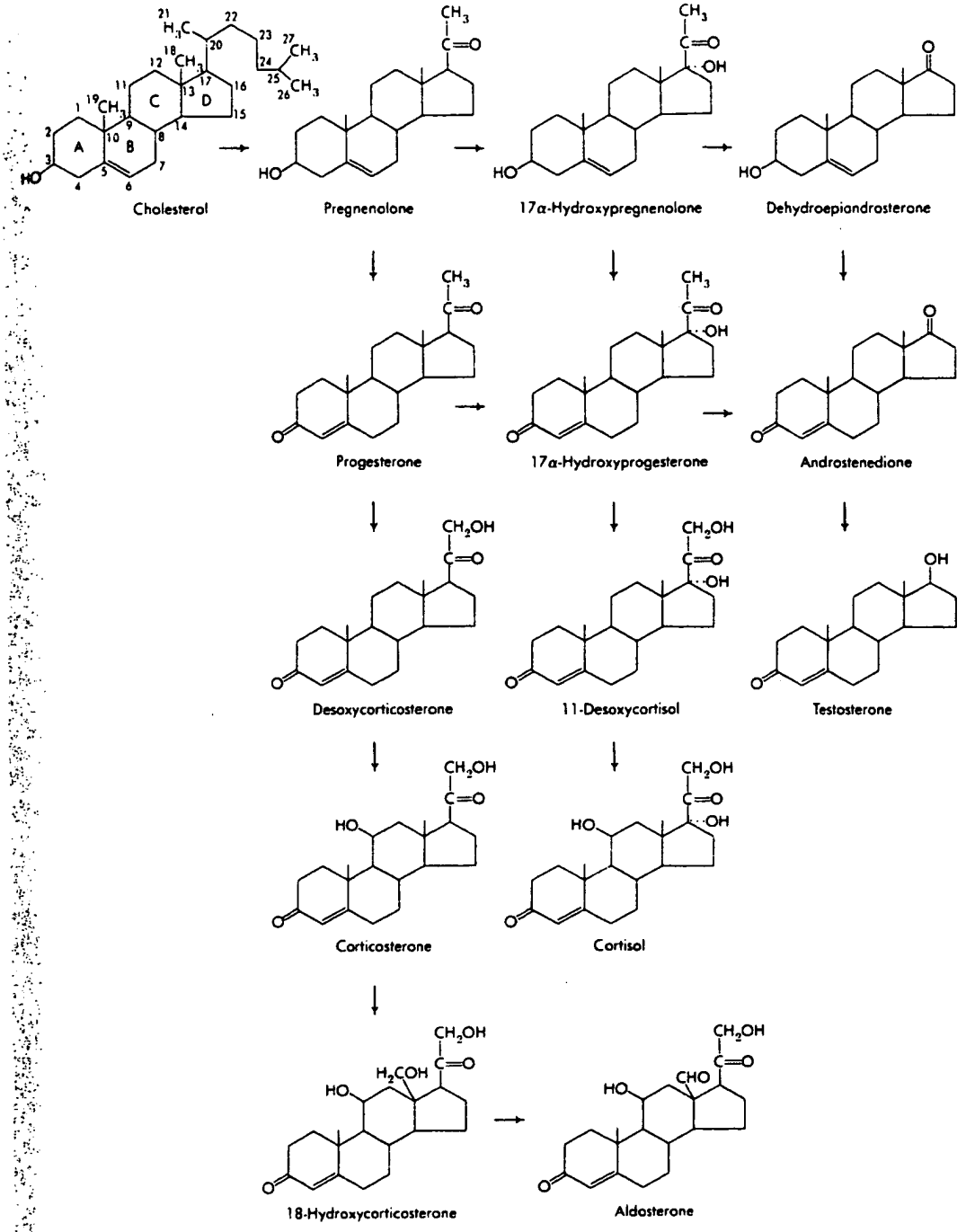


Figure 63–3. *Principal pathways for biosynthesis of adrenocorticosteroids and adrenal androgens.*

hypercorticism, that is, signs of excess of corticosteroid. The fluctuations in the secretory activity of a normal subject are presumed to reflect the varying needs of the organism for corticosteroids.

The actions of corticosteroids are often complexly related to the functions of other hormones. For example, in the absence of lipolytic hormones, cortisol even in large concentrations has virtually no effect on

the rate of lipolysis in adipose tissue *in vitro*. Likewise, a sympathomimetic amine has only slight effect on the rate of lipolysis if there is a deficiency of glucocorticoids. However, if a necessary minimal amount of cortisol is added, the lipolytic effect of the sympathomimetic amine becomes evident. The necessary but not sufficient role of corticosteroids acting in concert with other regulatory forces has been termed "permissive" by Ingle (1954).

Certain of the biological actions of the corticosteroids lend themselves to quantitative measurement. Estimates of the potencies of naturally occurring and synthetic corticosteroids in the categories of *sodium retention* (reduction of sodium excretion by the kidney of the adrenalectomized animal), *hepatic deposition of glycogen*, and *anti-inflammatory effect* (inhibition of the action of an agent that induces inflammation) are presented in Table 63–2. It should be noted that such values are not fixed ratios but vary considerably with the conditions of the bioassays used. Potencies of steroids as judged by ability to sustain life in the adrenalectomized animal closely parallel those determined for sodium retention. Potencies based on liver glycogen deposition, anti-inflammatory effect, work capacity of skeletal muscle, and involution of lymphoid tissue closely parallel one another. Dissociations exist between potencies based on sodium retention and on liver glycogen deposition; traditionally the corticosteroids have thus been classified into *mineralocorticoids* and *glucocorticoids*, according to potencies in the two categories. Desoxycorticosterone, the prototype of the mineralocorticoids, is highly potent in regard to sodium retention but without effect on hepatic glycogen deposition. Cortisol, the prototype of the glucocorticoids, is highly potent in regard to liver glycogen deposition but weak in regard to sodium retention. The naturally occurring corticosteroids cortisol and cortisone as well as synthetic corticosteroids such as prednisolone and triamcinolone are classified as glucocorticoids. However, corticosterone is a steroid that has modest but significant activities in both categories. In contrast, aldosterone is exceedingly potent with respect to sodium retention, with modest potency for liver glycogen deposition. At rates secreted by the adrenal cortex or in doses that exert maximal effects on electrolyte balance, aldosterone has no significant effect on carbohydrate metabolism; it is thus classified as a mineralocorticoid.

Table 63–2. RELATIVE POTENCIES OF CORTICOSTEROIDS

	SODIUM RETENTION	LIVER GLYCOGEN DEPOSITION	ANTI-INFLAMMATORY EFFECT
Natural Steroids			
Cortisol	1 *	1	1
Cortisone	0.8 *	0.8	0.8
Corticosterone	15	0.35	0.3
11-Desoxycorticosterone	100	0	0
Aldosterone	3000	0.3	?
Synthetic Steroids			
Prednisolone	<1 *	4	4
Triamcinolone	0	5	5

* Promotes sodium excretion under certain circumstances.

In the following descriptions of the physiological functions and the pharmacological effects of the corticosteroids, the terms *mineralocorticoid* and *glucocorticoid* will be employed for convenience. It is to be emphasized that the biological characteristics of the corticosteroids range over a spectrum from that of a strictly mineralocorticoid type at the one end to that of the strictly glucocorticoid type at the other. A comprehensive review of the actions of the glucocorticoids is that edited by Baxter and Rousseau (1979).

Mechanism of Action. Corticosteroids, like other steroid hormones, are thought to act by controlling the rate of synthesis of proteins. As with estrogens (Chapter 61), the corticosteroids react with receptor proteins in the cytoplasm of sensitive cells to form a steroid-receptor complex. Such receptors have been identified in many tissues (Ballard *et al.*, 1974). The steroid-receptor complex undergoes a modification, as noted by an increase in the sedimentation constant; following this, the complex moves into the nucleus, where it binds to chromatin. Information carried by the steroid or more likely by the receptor protein activates the genetic apparatus to transcribe RNA. This is established by demonstrations that glucocorticoids, in appropriate tissues, increase the synthesis of mRNA that codes for enzymes whose synthesis is stimulated by these hormones (Schutz *et al.*, 1975; Iynedjian and Hanson, 1977). A more precise understanding of the mechanism by which glucocorticoids activate transcription of specific mRNA

may soon be forthcoming. Glucocorticoids specifically stimulate the rate of viral gene transcription in cultured tumor cells that bear murine mammary tumor virus, and this provides an excellent model system for study of the action of these hormones. The steroid-receptor complex binds *in vitro* to specific sequences of the viral DNA (Payvar *et al.*, 1981). The segments of DNA that are recognized are the long terminal repeat sequences that are known to be sites where transcription is initiated (Govindan *et al.*, 1982).

Steroid hormones thus stimulate transcription and ultimately the synthesis of specific proteins. While this is true for corticosteroids in some tissues, such as the liver, in other tissues, for example, lymphoid cells, the overall effect of the hormones is a catabolic one. This suggests that the steroid-receptor complex may inhibit rather than stimulate transcription in these instances. However, Makman and coworkers (1971) presented evidence suggesting that steroids act in lymphatic cells to stimulate the synthesis of an inhibitory protein, which presumably causes the catabolic effects.

Carbohydrate and Protein Metabolism. The effects of adrenocortical hormones on carbohydrate and protein metabolism are epitomized in the teleological view that these steroids have evolved to protect glucose-dependent cerebral functions by stimulating the formation of glucose, diminishing its peripheral utilization, and promoting its storage as glycogen. Adrenalectomized animals exhibit no marked abnormality in carbohydrate metabolism if food is regularly available. Under such circumstances, normal concentrations of glucose in the plasma are maintained and glycogen is stored in the liver. However, a brief period of starvation rapidly depletes carbohydrate reserves. The concentration of glycogen in the liver, and to a lesser extent that in muscle, decreases and hypoglycemia develops. In light of these facts, it is not surprising that the adrenalectomized animal is hypersensitive to insulin. Patients with Addison's disease have similar abnormalities in carbohydrate metabolism.

Administration of a glucocorticoid such as cortisol corrects the defect in carbohydrate metabolism of the adrenalectomized animal: glycogen stores, particularly in the liver, are increased; concentrations of glucose in plasma remain normal during fasting; sensitivity to insulin returns to normal. Increased excretion of nitrogen accompanies the increased production of glucose, indicating that protein is converted to carbohydrate (Long *et al.*, 1940). Prolonged exposure to large doses of glucocorticoids leads to an exaggeration of these changes in glucose metabolism, so that a diabetic-like state is produced: glucose in the plasma tends to be elevated in the fasting subject, there is increased resistance to insulin, glucose tolerance is decreased, and glucosuria may be present.

The mechanism by which the glucocorticoids inhibit utilization of glucose in peripheral tissues is not understood. Decreased uptake of glucose has been demonstrated in adipose tissue, skin, fibroblasts, and thymocytes as a result of glucocorticoid action.

Glucocorticoids promote gluconeogenesis by both peripheral and hepatic actions. Peripherally these steroids act to mobilize amino acids from a number of tissues. This catabolic action of the glucocorticoids is reflected in the atrophy of lymphatic tissues, reduced mass of muscle, osteoporosis (reduction in protein matrix of bone followed by calcium loss), thinning of the skin, and a negative nitrogen balance. Amino acids funnel into the liver, where they serve as substrates for enzymes involved in the production of glucose and glycogen.

In the liver the glucocorticoids induce *de-novo* synthesis of a number of enzymes involved in gluconeogenesis and amino acid metabolism. For example, the hepatic enzymes phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase, which catalyze reactions of glucose synthesis, are increased in concentration. However, induction of these enzymes requires a matter of hours and cannot account for the earliest effects of the hormones on gluconeogenesis. More rapid effects of glucocorticoids are apparent on hepatic mitochondria, such that they carboxylate pyruvate to form oxaloacetate at an accelerated rate (Adam and Haynes, 1969). This is the first reaction in the synthesis of glucose from pyruvate.

Prolonged, but not acute, treatment with glucocorticoids has been found to elevate the concentration of glucagon in the plasma (Marco *et al.*, 1973; Wise *et al.*, 1973). Inasmuch as glucagon itself stimulates gluconeogenesis, the rise in glucagon should also contribute to the enhanced synthesis of glucose. The deposition of glycogen in the liver found after treatment with glucocorticoids is thought to be the consequence of activation of hepatic glycogen synthase. This activation requires the presence of insulin but is not mediated by a rise in the concentration of insulin (Vanstapel *et al.*, 1982).

Lipid Metabolism. Two effects of corticosteroids on lipid metabolism are firmly established. The first is the dramatic redistribution of body fat that occurs in the hypercorticoid state. The other is the facilitation of the effect of adipokinetic agents in eliciting lipolysis of the triglycerides of adi-

pose tissue. A number of other effects of corticosteroids on lipids have been reported, but in few, if any, instances have they turned out to be direct actions of the corticosteroids themselves.

Administration of large doses of glucocorticoids to human subjects over a long period of time or the hypersecretion of cortisol that occurs in Cushing's syndrome leads to a peculiar alteration in fat distribution. There is a gain of fat in depots in the back of the neck ("buffalo hump"), supraclavicular area, and face ("moon face") and a loss of fat from the extremities. One hypothesis to explain this phenomenon is that of Fain and Czech (1975), who proposed that the adipose tissue that hypertrophies in Cushing's syndrome responds preferentially to the lipogenic and antilipolytic actions of the elevated concentrations of insulin evoked by glucocorticoid-induced hyperglycemia. According to this hypothesis, adipocytes in the extremities, in contrast to those of the trunk, are less sensitive to insulin and more sensitive to the glucocorticoid-facilitated lipolytic effects of other hormones.

The mobilization of fat from peripheral fat depots by epinephrine, norepinephrine, or adipokinetic peptides of the adenohypophysis is markedly blunted in the absence of the adrenal cortex or the adenohypophysis. Cortisol acts in adipose tissue to facilitate the lipolytic response to cyclic AMP, rather than to enhance its accumulation. Hypophysectomy in rats has only a slight effect on the accumulation of cyclic AMP after exposure of adipose tissue to graded doses of epinephrine (Birnbaum and Goodman, 1973); however, hypophysectomy greatly decreases the lipolytic response of adipose tissue to the cyclic nucleotide. Treatment with cortisol restores the normal response to lipolytic hormones and to cyclic AMP (Goodman, 1968). Plasma lipids are not changed consistently in either hypocorticism or hypercorticism.

Electrolyte and Water Balance. Mineralocorticoids act on the distal tubules of the kidney to enhance the reabsorption of sodium ions from the tubular fluid into the plasma; they increase the urinary excretion of both potassium and hydrogen ions. The consequences of these three primary effects in concert with similar actions on cation transport in other tissues appear to account for the entire spectrum of physiological and pharmacological activities that are characteristic of the mineralocorticoids. Thus, the primary features of *hypercorticism* are positive sodium balance and expansion of the extracellular fluid volume, normal or slight increase in the concentration of sodium in the plasma, hypokalemia, and alkalosis. In contrast, those of the deficient state, *hypocorticism*, are sodium loss, hyponatremia, hyperkalemia, contraction of the extracellular fluid volume, and cellular hydration. A defect of major consequence in adrenocortical insufficiency is the renal loss of sodium. The renal tubules normally reabsorb practically all the sodium filtered at the glomerulus. For example, on an ordinary diet, 99.5% may be reabsorbed to maintain sodium balance. Typically, in a patient with Addison's disease under the same circumstances of dietary intake, maximal reabsorption attainable is 98.5%. Since approximately 24,000 mEq of sodium is filtered per day, the 1% difference between reabsorption in the normal subject and reabsorption in the patient with Addison's disease amounts to a loss of 240 mEq of sodium per day. The gravity of the situation is obvious when one considers that this amount of sodium is normally present in 1.7 liters of extracellular fluid. Proportionately more sodium than water is lost through the kidney and the concentration of extracellular sodium decreases; extracellular fluid becomes hypoosmotic, and water shifts from the extracellular into the intracellular compartment. This shift, together with the renal loss of water, results in a marked reduction in the volume of the extracellular fluid. Cells are hydrated, and the increase in the hematocrit value is due not only to a shrinkage of the plasma volume but also to the swelling of the erythrocytes. Hyperkalemia and the tendency toward acid-base disturbances are a result of impairments in the excretion of potassium and of hydrogen ions. Without administration of mineralocorticoids or sodium chloride solution or both, a rapid downhill course ensues in adrenocortical insufficiency. The shrinkage of extracellular fluid volume, the cellular hydration, and the hypodynamic state of the cardiovascular system combine to cause circulatory collapse, renal failure, and death.

In adrenocortical insufficiency, a basic defect in ion transport occurs in a variety of secretory cells. Not only the kidney but also the salivary glands, the sweat glands, the exocrine pancreas, and the mucosa of the gastrointestinal tract elaborate fluids abnormally high in the concentration of sodium and abnormally low in the concentration of potassium. In the patient with Addison's disease, sweating may contribute significantly to the negative balance of sodium.

Aldosterone is by far the most potent of the naturally occurring corticosteroids with regard to electrolyte balance and plays an important role in the regulation of sodium and potassium balance. Evidence of this is the relatively normal electrolyte balance exhibited by the hypophysectomized animal as a result of continued secretion of aldosterone by the adrenal cortex. The increased rate of secretion of aldosterone that occurs in man when dietary salt is severely limited would appear to be a compensatory adjustment of physiological importance. However, *changes* in the rate of secretion of aldosterone are not the cause of *rapid* changes that may occur in sodium excretion. The latent period of action of the steroid is too long.

The intravenous administration of aldosterone to a normal subject is followed, after a delay of about an hour, by a decrease in the rate of renal sodium ion excretion and an increase in the rate of potassium ion and hydrogen ion excretion. If the administration of relatively large amounts of aldosterone is continued over a period of more than 10 to 14 days, sodium excretion again equals sodium intake. However, potassium ion and hydrogen ion excretion continues at an accelerated rate, resulting in hypokalemic hypochloremic alkalosis. The mechanism of "escape" from acute sodium retention is not understood, but it is not due to suppression of the renin-angiotensin system. The effects of the mineralocorticoids have been reviewed by Mulrow and Forman (1972).

The morphological complexity of the mammalian kidney presents a formidable obstacle to an attack on the question of how aldosterone increases sodium reabsorption. Aldosterone stimulates sodium transport by the toad bladder, and it is understandable that investigators have turned to this structurally simple organ as an experimental system.

Studies with the toad bladder have indicated that aldosterone, like other steroids, probably acts to initiate transcription of RNA that serves as template for the synthesis of a protein or proteins. This hypothetical "aldosterone-induced protein" is thought to facilitate the transport of sodium ions from the lumen of the distal tubules through the tubular cells and into the extracellular fluid. The most widely accepted model to describe the action of aldosterone is the following (Marver, 1980). The sodium ions of the tubular filtrate enter the cells of the distal tubules down a concentration gradient through the cell membrane facing the tubular lumen (apical or mucosal surface). Aldosterone and other mineralocorticoids facilitate this diffusion by increasing the permeability of the apical membrane to sodium ions. Sodium ions therefore enter the cells at an accelerated rate and are pumped out into the extracellular space at the serosal surface by a Na^+,K^+-activated adenosine triphosphatase (Na^+,K^+-ATPase).

The mechanisms of the enhanced excretion of potassium and hydrogen ions are less well understood. For practical purposes one may visualize these ions as being "exchanged" for the additional sodium ions reabsorbed under the influence of the steroids, because the sum of the equivalents of the additional potassium and hydrogen ions excreted is equal to that of the additional sodium ions retained.

The *glucocorticoids* decrease the absorption of calcium from the intestine and increase its renal excretion, thus producing a negative balance of the cation. These effects are considered to be the basis of the favorable therapeutic response to glucocorticoids seen in hypercalcemia (*see* Chapter 65).

Desoxycorticosterone is a natural mineralocorticoid of some historical interest for it was the first corticosteroid to be synthesized and made available for the treatment of Addison's disease. Desoxycorticosterone is practically devoid of glucocorticoid effects. Qualitatively, it is identical to aldosterone in its effects on electrolytes; quantitatively, it is about 3% as potent (*see* Table 63–2). Thus, despite the fact that the concentration of desoxycorticosterone in plasma is approximately the same as that of aldosterone, it apparently is of little physiological significance in the normal individual (Biglieri, 1978).

Cortisol induces sodium retention and potassium excretion, but much less effectively than does aldosterone. Acute treatment with cortisol, unlike that with aldosterone, does not increase net acid secretion (Lemann *et al.*, 1970). In striking contrast to aldosterone, cortisol, under certain circumstances, especially sodium loading, enhances sodium excretion. This may be accounted for by the capacity of cortisol to increase the glomerular filtration rate (GFR). Aldosterone and desoxycorticosterone are ineffective in this regard. Fur-

thermore, cortisol has a significant stimulatory influence on tubular secretory activity.

Impaired water diuresis in response to an administered water load, while not specific for adrenal insufficiency, has been used as a diagnostic criterion. In adrenal insufficiency, GFR is reduced and plasma antidiuretic hormone (ADH) concentration is increased; these factors account for failure to excrete a water load (Ahmed *et al.*, 1967). Administration of cortisol, but not of aldosterone, increases GFR and restores water diuresis (Gill *et al.*, 1962).

Hypercorticism due to administration of large doses of cortisol (or related glucocorticoids) or to excessive secretion of cortisol by the adrenals is sometimes associated with a hypokalemic hypochloremic alkalosis (*see* Chapter 35). However, the changes, particularly the degree of hypokalemia, are moderate in severity and reflect the relatively weak effect of cortisol as compared to aldosterone on electrolyte balance. Muscular weakness associated with glucocorticoid treatment is usually due to a loss of muscle mass rather than of potassium.

Cardiovascular System. The most striking effects of corticosteroids on the cardiovascular system are those that are the consequence of regulation of renal sodium ion excretion. These are seen most vividly in hypocorticism when reduction in blood volume accompanied by increased viscosity can lead to hypotension and cardiovascular collapse. However, the impairment of the cardiovascular system in adrenocortical insufficiency obviously involves additional, poorly understood processes. The corticosteroids exert important actions on the various elements of the circulatory system, including the capillaries, the arterioles, and the myocardium. In the absence of the corticosteroids, there is increased capillary permeability, inadequate vasomotor response of the small vessels, and reduction in cardiac size and output.

An excess of mineralocorticoids occurs in its purest form in *primary aldosteronism*, the result of excessive secretion of this steroid. In this disease the major clinical findings are hypertension and hypokalemia. The hypokalemia is an obvious consequence of the renal effects of aldosterone, but the genesis of the hypertension has not been totally clarified. Development of hypertension requires a prolonged excess of mineralocorticoid and increased sodium intake (Mulrow and Forman, 1972). Hypertension occurs in most cases of Cushing's syndrome but rarely, if at all, as the result of administration of synthetic glucocorticoids lacking mineralocorticoid activity. Steroid-induced hypertension may be the result of prolonged, excessive sodium retention; one hypothesis proposes that this leads to edema within the walls of arterioles, thereby reducing their lumina and increasing peripheral vascular resistance (Tobian, 1960). Another possibility is that salt retention or mineralocorticoids themselves sensitize blood vessels to pressor agents, in particular angiotensin and catecholamines (Brunner *et al.*, 1972; Yard and Kadowitz, 1972). The concentration of renin substrate is elevated in Cushing's syndrome, and this too may play a role (Krakoff *et al.*, 1975). There is also some evidence that ADH plays a role in the pathogenesis of hypertension produced by mineralocorticoids (Share and Crofton, 1982).

Skeletal Muscle. The maintenance of normal function of skeletal muscle requires adequate concentrations of corticosteroids, but excessive amounts of either mineralocorticoids or glucocorticoids lead to abnormalities.

It is well known that one of the outstanding signs of adrenocortical insufficiency is a diminished work capacity of striated muscle. This is manifested in patients with Addison's disease by weakness and fatigue. The most important single factor responsible for this dysfunction appears to be the inadequacy of the circulatory system. Abnormalities in electrolyte balance and carbohydrate metabolism in adrenocortical insufficiency contribute only in small measure to the impairment in skeletal muscle function.

Muscle weakness in primary aldosteronism is in large measure a result of the hypokalemia characteristic of this disease. Glucocorticoids given for prolonged periods in high doses or secreted in abnormal amounts in Cushing's syndrome tend to cause a wasting of skeletal muscle. The mechanism of this is not known. This steroid myopathy is responsible, at least in part, for the weakness and fatigue noted in the syndrome. Steroid-induced myopathy has been reviewed by Mandel (1982).

Central Nervous System. The corticosteroids affect the central nervous system (CNS) in a number of indirect ways; in particular, they maintain normal concentrations of glucose in plasma, an adequate circulation, and the normal balance of electrolytes in the body. The steroids may also have direct effects, but these are as yet poorly defined. An influence of the corticosteroids can be observed on mood, behavior, the EEG, and brain excitability.

Patients with Addison's disease exhibit apathy, depression, and irritability, and some are frankly psychotic. Desoxycorticosterone is ineffective but cortisol is very effective in correcting these abnormalities of psyche and behavior. An array of reactions, varying in degree and kind, is seen in patients to whom glucocorticoids are administered for therapeutic purposes. Most patients respond with elevation in mood, which may be explained in part by the relief of the symptoms of the disease being treated. In some, more definite mood changes occur, characterized by euphoria, insomnia, restlessness, and increased motor activity. A smaller but significant percentage of patients treated with high doses of cortisol become anxious or depressed, and a still smaller percentage exhibit psychotic reactions. There is a high incidence of neuroses and psychoses among patients with Cushing's syndrome. The abnormalities of behavior usually disappear when the corticosteroids are withdrawn or the Cushing's syndrome is effectively treated.

There is usually an increase in the excitability of neural tissue in hypocorticism and a decrease in animals given large doses of desoxycorticosterone; these alterations appear to be related to changes in the concentrations of electrolytes in the brain. In contrast, administration of cortisol increases brain excitability without influencing the concentrations of sodium and potassium in the brain. It is concluded that the influence of desoxycorticosterone on excitability is mediated through its influence on sodium transport, whereas cortisol acts by a different mechanism, presumably mediated by cytoplasmic receptors (McEwen, 1979; Carpenter and Gruen, 1982).

Thresholds for the perception of taste, smell, and sound stimuli are reduced in adrenocortical insufficiency and elevated in hypercorticism. Glucocorticoids restore thresholds to normal, but desoxycorticosterone is without effect (Henkin, 1970).

Formed Elements of Blood. Glucocorticoids tend to increase the hemoglobin and red-cell content of the blood, as evidenced by the frequent occurrence of polycythemia in Cushing's syndrome and a mild, normochromic, normocytic anemia in Addison's disease. The capacity of these steroids to retard erythrophagocytosis may be a factor in the production of polycythemia.

The corticosteroids also affect circulating white cells. Administration of glucocorticoids leads to an increase in the number of polymorphonuclear leukocytes in the blood as the result of an increased rate of entrance into the blood from the marrow and a diminished rate of removal from the circulation (Bishop *et al.*, 1968). In contrast, the lymphocytes, eosinophils, monocytes, and basophils of the blood decrease in number after administration of glucocorticoids. A single dose of cortisol produces a decline of about 70% in circulating lymphocytes and a decline of over 90% in monocytes; this occurs in 4 to 6 hours and lasts for about 24 hours. The decrease in lymphocytes, monocytes, and eosinophils appears to result from redistribution of cells, rather than from their destruction. The cause of the fall in circulating basophils has not been established.

After administration of a glucocorticoid, the thymus-derived lymphocytes (T cells) are decreased proportionately more than those that are derived from the bone marrow (B cells). The profile of cellular responses of the lymphocytes remaining in the blood to various mitogens and antigens is altered when contrasted to that of lymphocytes of untreated subjects. This indicates that subpopulations of lymphocytes are differentially affected by the steroids (*see* Cupps and Fauci, 1982).

Anti-inflammatory Properties. Cortisol and the synthetic analogs of cortisol have the capacity to prevent or suppress the development of the local heat, redness, swelling, and tenderness by which inflammation is recognized. At the microscopic level, they inhibit not only the early phenomena of the inflammatory process (edema, fibrin deposition, capillary dilatation, migration of leukocytes into the inflamed area, and phagocytic activity) but also the later manifestations (capillary proliferation, fibroblast proliferation, deposition of collagen, and, still later, cicatrization).

Although understanding of these effects is unsatisfactory, many observations have been made that have therapeutic relevance and that must be taken into account in explanatory formulations. Perhaps the most important of these for the physician is that corticosteroids inhibit the inflammatory response whether the inciting agent is radiant, mechanical, chemical, infectious, or immunological. In clinical terms, the administration of corticosteroids for their anti-inflammatory effects is palliative therapy; the underlying cause of the disease remains; the inflammatory manifestations are merely suppressed. It is this suppression of inflammation and its consequences that has made the corticosteroids such valuable therapeutic agents—indeed, at times lifesaving. It is also this property that gives them a nearly unique potential for therapeutic disaster. The signs and symptoms of inflammation are expressions of the disease process that are often used by the physician in diagnosis and in evaluating the effectiveness of treatment. These may be missing in patients treated with glucocorticoids. For example, an infection may continue to progress while the patient superficially appears to improve, and a peptic ulcer may perforate without producing clinical signs. This situation has been epitomized in the grimly facetious remark that the corticosteroids, misused, permit a patient to walk all the way to the autopsy room!

Anti-inflammatory effects depend upon the direct local action of the steroids. The most important factor in the anti-inflammatory action of gluco-

corticoids may be their ability to inhibit the recruitment of neutrophils and monocyte-macrophages into the affected area (Parrillo and Fauci, 1979). Treatment with glucocorticoids decreases the adherence of neutrophils to nylon fibers, and this may reflect a diminished tendency of these cells to adhere to capillary endothelial cells in areas of inflammation (MacGregor, 1977).

In the inflammatory response of delayed sensitivity reactions, lymphocytes previously sensitized to a particular antigen encounter the antigen within a tissue at a site destined to be the location of the inflammatory response. These lymphocytes, activated by the antigen, begin production of a number of soluble factors, *lymphokines*, that control the cellular response. Among the lymphokines is the macrophage migration inhibitory factor (MIF), which causes an accumulation of nonsensitized macrophages in the area by inhibiting their mobility (Bloom and Bennett, 1966). Glucocorticoids do not affect the production of MIF by lymphocytes that have been activated by an appropriate antigen, but the steroids do block the effect of MIF on macrophages; that is, the movement of these cells is no longer impeded, and they do not accumulate locally (Balow and Rosenthal, 1973).

Low concentrations of glucocorticoids inhibit the formation of plasminogen activator by neutrophils (Granelli-Piperano *et al.*, 1977). This enzyme converts plasminogen to plasmin (fibrinolysin), which is thought to facilitate the entrance of leukocytes into areas of inflammation by hydrolysis of fibrin and other proteins. There is also substantial evidence that the glucocorticoids induce the synthesis of a protein that inhibits phospholipase A_2 and thereby diminishes release of arachidonic acid from phospholipids. This decreases formation of prostaglandins, leukotrienes, and related compounds, such as prostaglandin endoperoxides and thromboxane, which may play an important role in chemotaxis and inflammation (Blackwell *et al.*, 1980; Hirata *et al.*, 1980; *see* Chapter 28). As a result of studies on a particular strain of mouse fibroblasts maintained in culture, it was thought that glucocorticoids inhibit the growth of such cells. It is now evident that human fibroblasts are resistant to glucocorticoids; the suppression of late stages of inflammation by inhibition of fibroblast proliferation is probably not a valid model for the anti-inflammatory effects of glucocorticoids (Priestley and Brown, 1980).

Lymphoid Tissue and Immune Responses. Addison was the first to observe the increase in mass of lymphoid tissue that accompanies adrenocortical insufficiency; there is also a lymphocytosis. In contrast, Cushing's syndrome is characterized by lymphocytopenia and decreased mass of lymphoid tissue.

While glucocorticoids cause a rapid lysis of lymphatic tissue in rats and mice, there is no evidence of a comparable effect in man (Claman, 1972). This implies that the changes in lymphoid tissue seen in man in chronic hypercorticosteroid or hypocorticosteroid states must result from changes in rates of cellular formation or destruction that become manifest over a prolonged period of time. As noted above, the acute effects of steroids on circulating lymphocytes are due to sequestration from the blood rather than to lymphocytolysis. Although glucocorticoids do not produce a sudden massive lysis of lymphoid tissue in man, cells of acute lymphoblastic leukemia and, in some cases, cells of other lymphatic malignancies are destroyed by glucocorticoids in a manner presumed to be analogous to that which occurs in lymphoid tissue of rodents.

As noted, glucocorticoids and ACTH modify the clinical course of a variety of diseases in which hypersensitivity is believed to play an important role. Although massive doses of methylprednisolone have been shown to cause a modest fall in the concentration of IgG in the plasma of human volunteers, these same subjects produced antibody normally in response to antigenic stimuli (Butler, 1975). On the whole, there is no convincing evidence that the therapeutic use of the corticosteroids has a significant effect on the titer of circulating antibodies, either IgG or IgE, that play a major role in allergic and autoimmune states. Metabolism of complement is likewise probably not significantly affected (Claman, 1975). This is true in spite of the fact that the symptoms of the diseases are often alleviated dramatically by the steroids. It is also now believed that in clinical situations in which the glucocorticoids are used to prevent the consequences of cell-mediated (delayed hypersensitivity) immune reactions, for example, graft rejection, the steroids do not interfere with the development of immune lymphatic cells that are capable of eliciting an inflammatory response upon contact with the sensitizing antigen. Rather, they suppress the inflammatory response, apparently by inhibiting recruitment of leukocytes into the region of contact with the foreign antigen (*see* above; Weston *et al.*, 1973).

Circulating monocytes from individuals who are receiving glucocorticoids display an impaired ability to kill microorganisms, although the process of phagocytosis is not defective (Rinehart *et al.*, 1975).

There are numerous reports of effects of glucocorticoids on lymphatic cells *in vitro*. Unfortunately, many of these effects are observed at unrealistically high concentrations of steroids, and their significance is unclear. One example of a response that is inhibited by appropriately low concentrations of glucocorticoids *in vitro* is the proliferation of T cells stimulated by mitogens or mixed leukocyte cultures. This effect is the result of inhibition of the release of interleukin 1 by macrophages. Deficiency of interleukin 1 precludes formation of interleukin 2, the immediate stimulus for proliferation of T cells (Gillis *et al.*, 1979; Smith, 1980).

Growth and Cell Division. Pharmacological doses of glucocorticoids retard or interrupt the growth of children, indicating an adverse effect on the epiphyseal cartilage. Inhibition of growth is a rather widespread effect of the glucocorticoids in tissues of laboratory animals. For example, they inhibit cell division or the synthesis of DNA in thymocytes; normal, developing, and regenerating liver; gastric mucosa; developing brain; developing lung; and human epidermis. Nevertheless, this effect is somewhat selective, and corticosteroids do not characteristically produce the bone-marrow depression or the enteritis that follows exposure to nonspecific antimitotic agents. The mechanism of this effect of the steroids is not known.

ABSORPTION, TRANSPORT, METABOLISM, AND EXCRETION

Absorption. Cortisol and numerous congeners, including synthetic analogs, are effective when given by mouth. Desoxycorticosterone acetate is unusual in that it is ineffective by this route.

Water-soluble esters of cortisol and its synthetic congeners are administered intravenously in order to achieve high concentrations in body fluids rapidly. More prolonged effects are obtained by intramuscular injection of suspensions of cortisol, congeners, and esters. Minor changes in chemical structure may result in large changes in the rate of absorption, time of onset of effect, and duration of action.

Glucocorticoids are absorbed from sites of local application such as synovial spaces, the conjunctival sac, and the skin. The absorption may be sufficient, when administration is chronic or large areas of skin are involved, to cause systemic effects, including adrenocortical suppression.

Transport, Metabolism, and Excretion. In the plasma, 90% or more of the cortisol is reversibly bound to protein under normal circumstances. The binding is accounted for by two proteins. One, corticosteroid-binding globulin, is a glycoprotein; the other is albumin. The globulin has high affinity but low total binding capacity, while albumin has low affinity but relatively large binding capacity. Consequently, at low or normal concentrations of corticosteroids most of the hormone is bound to globulin. When the amount of corticosteroid is increased, concentrations of both free and albumin-bound steroid increase with little change in the concentration of that bound to the globulin. Corticosteroids compete with each other for binding sites on the corticosteroid-binding globulin. Cortisol has high affinity; glucuronide-conjugated steroid metabolites and aldosterone have low affinities.

During pregnancy and during estrogen treatment in both sexes, corticosteroid-binding globulin, total plasma cortisol, and free cortisol increase several-fold. The physiological significance of these facts is not known. The free hormone as opposed to the protein-bound steroid is biologically active, available for hepatic metabolism, and may be excreted by the kidney.

All the biologically active adrenocortical steroids and their synthetic congeners have a double bond in the 4,5 position and a ketone group at C 3. Reduction of the 4,5 double bond can occur at both hepatic and extrahepatic sites and yields an inactive substance. Subsequent reduction of the 3-ketone substituent to a 3-hydroxyl to form tetrahydrocortisol has been demonstrated only in liver. Most of the ring-A–reduced metabolites are enzymatically coupled through the 3-hydroxyl with sulfate or with glucuronic acid to form water-soluble sulfate esters or glucuronides, and they are excreted as such. These conjugation reactions occur principally in liver and to some extent in kidney.

Reversible oxidation of the 11-hydroxyl group has been demonstrated to occur slowly in a variety of extrahepatic tissues and rapidly in liver. Corticosteroids with an 11-ketone substituent require reduction to 11-hydroxyl compounds for their biological

activity. Reduction of the 20-ketone group to a 20-hydroxyl configuration yields a substance having little, if any, biological activity. Corticosteroids with a hydroxyl group at C 17 undergo an oxidation that yields 17-ketosteroids and a two-carbon fragment. These 17-ketosteroids are totally lacking in corticosteroid activity but, in a few instances, have weak androgenic properties.

When radioactive-carbon, ring-labeled steroids are injected intravenously in man, most of the radioisotope is recovered in the urine within 72 hours. Neither biliary nor fecal excretion is of any quantitative importance in man. It has been estimated that the liver metabolizes at least 70% of the cortisol secreted.

The metabolism of cortisol has been studied more extensively than that of all other corticosteroids, and it is generally assumed that the metabolism of its congeners and synthetic derivatives is qualitatively similar. Cortisol has a plasma half-life of about 1.5 hours. The metabolism of corticosteroids is greatly slowed by introduction of the 1,2 double bond or a fluorine atom into the molecule, and the half-life is correspondingly prolonged.

Clinical laboratories measure urinary cortisol and metabolites with reduced ring A as "17-hydroxycorticosteroids." These compounds and those where the ketone at carbon 20 has been reduced are included in the group referred to as "17-ketogenic steroids." The urinary metabolites that have lost their side chain contribute to the "17-ketosteroids."

STRUCTURE-ACTIVITY RELATIONSHIP

Cortisone was the first corticosteroid used for its anti-inflammatory effect. Modifications of structure have led to increases in the ratio of anti-inflammatory to sodium-retaining potency, such that in a number of presently available compounds electrolyte effects are of no serious consequence, even at the highest doses used. However, in all compounds studied to date, effects on inflammation and on carbohydrate and protein metabolism have paralleled one another. It seems very likely that effects on inflammation and metabolism are mediated by the same type of receptor.

Changes in molecular structure may bring about changes in biological potency as a result of alterations in absorption, protein binding, rate of metabolic transformation, rate of excretion, ability to traverse membranes, and intrinsic effectiveness of the molecule at its site of action. In the following paragraphs, modifications of the pregnane nucleus that have been of value in therapeutic agents are described. The molecular sites of alteration are shown in Figure 63–4 in bold lines and letters. Table 63–3 lists the effects of the modifications discussed relative to cortisol. As indicated above, relative potencies vary to some extent with different conditions of bioassay.

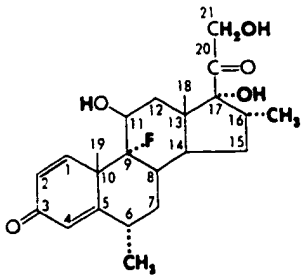


Figure 63–4. *Structure-activity relationship of adrenocorticosteroids.*

Light lines and letters indicate structural features common to compounds having anti-inflammatory action. Bold lines and letters indicate modifications that enhance or suppress characteristic activities. (After Liddle, 1961. Courtesy of *Clinical Pharmacology and Therapeutics*.)

Ring A. The 4,5 double bond and the 3-ketone are both necessary for typical adrenocorticosteroid activity. Introduction of a 1,2 double bond, as in prednisone or prednisolone, enhances the ratio of carbohydrate-regulating potency to sodium-retaining potency by selectively increasing the former. In addition, prednisolone is metabolized more slowly than cortisol.

Ring B. 6α-Substitution has unpredictable effects. In the particular instance of cortisol, 6α-methylation increases anti-inflammatory, nitrogen-wasting, and sodium-retaining effects in man. In contrast, 6α-methylprednisolone has slightly greater anti-inflammatory potency and less electrolyte-regulating potency than prednisolone. Fluorination in the 9α position enhances all biological activities of the corticosteroids, apparently by its electron-withdrawing effect on the 11β-hydroxy group.

Ring C. The presence of an oxygen function at C 11 is indispensable for significant anti-inflammatory and carbohydrate-regulating potency (cortisol versus 11-desoxycortisol) but is not necessary for high sodium-retaining potency, as demonstrated by desoxycorticosterone.

Ring D. 16-Methylation or hydroxylation eliminates the sodium-retaining effect but only slightly modifies potency with respect to effects on metabolism and inflammation.

All presently used anti-inflammatory steroids are 17α-hydroxy compounds. Although some carbohydrate-regulating and anti-inflammatory effects may occur in 17-desoxy compounds (cortisol versus corticosterone), the fullest expression of these activities requires the presence of the 17α-hydroxy substituent.

All natural corticosteroids and most of the active synthetic analogs have a 21-hydroxy group. While some glycogenic and anti-inflammatory activities may occur in its absence, its presence is required for significant sodium-retaining activity.

Table 63–3. RELATIVE POTENCIES AND EQUIVALENT DOSES OF CORTICOSTEROIDS

COMPOUND	RELATIVE ANTI-INFLAMMATORY POTENCY	RELATIVE SODIUM-RETAINING POTENCY	DURATION OF ACTION *	APPROXIMATE EQUIVALENT DOSE † (*mg*)
Cortisol (Hydrocortisone)	1	1	S	20
Tetrahydrocortisol	0	0	—	—
Prednisone (Δ^1-Cortisone)	4	0.8	I	5
Prednisolone (Δ^1-Cortisol)	4	0.8	I	5
6α-Methylprednisolone	5	0.5	I	4
Fludrocortisone (9α-Fluorocortisol)	10	125	S	—
11-Desoxycortisol	0	0	—	—
Cortisone (11-Dehydrocortisol)	0.8	0.8	S	25
Corticosterone	0.35	15	S	—
Triamcinolone (9α-Fluoro-16α-hydroxyprednisolone)	5	0	I	4
Paramethasone (6α-Fluoro-16α-methylprednisolone)	10	0	L	2
Betamethasone (9α-Fluoro-16β-methylprednisolone)	25	0	L	0.75
Dexamethasone (9α-Fluoro-16α-methylprednisolone)	25	0	L	0.75

* S = Short or 8- to 12-hour biological half-life; I = intermediate or 12- to 36-hour biological half-life; L = long or 36- to 72-hour biological half-life (*see* Rose and Saccar, 1978).

† These dose relationships apply only to oral or intravenous administration; relative potencies may differ greatly when injected intramuscularly or into joint spaces.

PREPARATIONS AND ROUTES OF ADMINISTRATION

Organic chemists have synthesized a bewildering number of modified adrenocorticosteroids, many of which share the same properties and differ only with respect to absolute dosage. At the outset it should be reemphasized that, whereas a clear separation has been made between mineralocorticoids and glucocorticoids, there is no member of the latter group that is unique with respect to a separation of therapeutic and toxic effects. A working knowledge of a small number of preparations is sufficient for nearly every clinical purpose.

Corticosteroids are administered orally, parenterally (intravenous, intramuscular, subcutaneous, intrasynovial, and intralesional routes), and topically (dermal ointments, creams, and lotions; ophthalmic ointments and solutions; respiratory aerosols; enemas). Some absorption into the systemic circulation occurs with all forms of topical administration. In the case of most aerosols, absorption is virtually equivalent to that from parenteral or oral administration. Adrenocortical suppression can occur with applications of steroids to the conjunctival sac and to the skin. Absorption from the skin is especially marked when the steroid is applied under plastic film over a large surface area.

Information on available steroid preparations is presented in Table 63–4.

TOXICITY OF ADRENOCORTICAL STEROIDS

Two categories of toxic effects are observed in the therapeutic use of adrenocorticosteroids: those resulting from *withdrawal* and those resulting from *continued use of large doses*. Acute adrenal insufficiency results from too rapid withdrawal of corticosteroids after prolonged therapy. Protocols for discontinuing corticosteroid therapy in patients who have been subjected to suppressive therapy for long periods have been described by Harter and associates (1963) and Byyny (1976). There is a characteristic corticosteroid withdrawal syndrome, consisting in fever, myalgia, arthralgia, and malaise, which may be extremely difficult to distinguish from "reactivation" of rheumatoid arthritis or rheumatic fever (Amatruda *et al.*, 1960). Pseudotumor cerebri with papilledema is a rare reaction that follows reduction or withdrawal of corticosteroid therapy (Levine and Leopold, 1973).

Table 63–4. PREPARATIONS OF ADRENOCORTICAL STEROIDS AND THEIR SYNTHETIC ANALOGS *

NONPROPRIETARY NAME AND TRADE NAMES	ORAL FORMS	INJECTABLE FORMS	OTHERS [1]
Desoxycorticosterone acetate [2] (DOCA ACETATE, PERCORTEN ACETATE)	—	5 mg/ml (oil) 125 mg (pellets)	—
Desoxycorticosterone pivalate (PERCORTEN PIVALATE)	—	25 mg/ml (susp.)	—
Fludrocortisone acetate [3] (FLORINEF ACETATE)	0.1 mg	—	—
Cortisol [2] (hydrocortisone) (CORTEF, HYDROCORTONE)	5–20 mg	25, 50 mg/ml (susp.)	TA: 0.125–2.5% 100-mg/60-ml enema
Cortisol (hydrocortisone) acetate (CORTEF ACETATE, HYDROCORTONE ACETATE, others)	—	25, 50 mg/ml (susp.)	TA: 0.5–2.5% 15-, 25-mg suppositories 10% rectal foam
Cortisol (hydrocortisone) cypionate (CORTEF FLUID)	2 mg/ml (susp.)	—	—
Cortisol (hydrocortisone) sodium phosphate (HYDROCORTONE PHOSPHATE)	—	50 mg/ml	—
Cortisol (hydrocortisone) sodium succinate (A-HYDROCORT, SOLU-CORTEF)	—	100–1000 mg (powder)	—
Beclomethasone dipropionate [4] (BECLOVENT, VANCERIL)	—	—	I: 42 μg per dose
Betamethasone [3] (CELESTONE)	0.6 mg 0.6 mg/5 ml (syrup)	—	—
Betamethasone benzoate (BENISONE, UTICORT)	—	—	TA: 0.025%
Betamethasone dipropionate (DIPROSONE)	—	—	TA: 0.05, 0.1%
Betamethasone sodium phosphate and acetate (CELESTONE SOLUSPAN)	—	6 mg/ml (susp.)	—
Betamethasone valerate (BETA-VAL, VALISONE)	—	—	TA: 0.01, 0.1%
Cortisone acetate [3] (CORTONE ACETATE)	5–25 mg	25, 50 mg/ml (susp.)	—
Dexamethasone [3] (DECADRON, others)	0.25–6.0 mg 0.5 mg/5 ml (elixir) 0.5 mg/0.5 ml (soln.)	—	TA: 0.01, 0.1% O: 0.1%
Dexamethasone acetate (DECADRON-LA, others)	—	2–16 mg/ml (susp.)	—
Dexamethasone sodium phosphate (DECADRON PHOSPHATE, HEXADROL PHOSPHATE, others)	—	4–24 mg/ml	TA: 0.1% O: 0.05, 0.1% I: 100 μg per dose
Methylprednisolone [3] (MEDROL)	2–32 mg	—	—

Table 63–4. PREPARATIONS OF ADRENOCORTICAL STEROIDS AND THEIR SYNTHETIC ANALOGS * (Continued)

NONPROPRIETARY NAME AND TRADE NAMES	ORAL FORMS	INJECTABLE FORMS	OTHERS [1]
Methylprednisolone acetate (DEPO-MEDROL, MEDROL ACETATE, others)	—	20–80 mg/ml (susp.)	TA: 0.25, 1% 40-mg/unit enema
Methylprednisolone sodium succinate (A-METHAPRED, SOLU-MEDROL)	—	40–1000 mg (powder)	—
Paramethasone acetate [3] (HALDRONE)	1, 2 mg	—	—
Prednisolone [3] (DELTA-CORTEF, others)	1, 5 mg	—	—
Prednisolone acetate (ECONOPRED, others)	—	25–100 mg/ml (susp.)	O: 0.12–1%
Prednisolone sodium phosphate (HYDELTRASOL, others)	—	20 mg/ml	O: 0.125–1%
Prednisolone tebutate (HYDELTRA-T.B.A.)	—	20 mg/ml (susp.)	—
Prednisone [3] (DELTASONE, others)	1–50 mg 1 mg/ml (syrup)	—	—
Triamcinolone [3] (ARISTOCORT, KENACORT)	1–16 mg	—	—
Triamcinolone acetonide (KENALOG, others)	—	10, 40 mg/ml (susp.)	TA: 0.025–0.5% I: 100 μg per dose
Triamcinolone diacetate (ARISTOCORT, KENACORT DIACETATE, others)	2, 4 mg/5 ml (syrup)	25, 40 mg/ml (susp.)	—
Triamcinolone hexacetonide (ARISTOSPAN)	—	5, 20 mg/ml (susp.)	—
Amcinonide [4] (CYCLOCORT)	—	—	TA: 0.1%
Clocortolone pivalate [4] (CLODERM)	—	—	TA: 0.1%
Desonide [4] (TRIDESILON)	—	—	TA: 0.05%
Desoximetasone [4] (TOPICORT)	—	—	TA: 0.05, 0.25%
Diflorasone diacetate [4] (FLORONE, MAXIFLOR)	—	—	TA: 0.05%
Flumethasone pivalate [4] (LOCORTEN)	—	—	TA: 0.03%
Fluocinolone acetonide [4] (FLUONID, SYNALAR, others)	—	—	TA: 0.01–0.2%
Fluocinonide [4] (LIDEX)	—	—	TA: 0.05%
Fluorometholone [4] (FML LIQUIFILM)	—	—	O: 0.1%

Table 63–4. PREPARATIONS OF ADRENOCORTICAL STEROIDS AND THEIR SYNTHETIC ANALOGS * (Continued)

NONPROPRIETARY NAME AND TRADE NAMES	ORAL FORMS	INJECTABLE FORMS	OTHERS [1]
Flurandrenolide [4] (CORDRAN)	—	—	TA: 0.025, 0.05% 4 μg/sq cm tape
Halcinonide [4] (HALOG)	—	—	TA: 0.025, 0.1%
Medrysone [4] (HMS LIQUIFILM)	—	—	O: 1%

* Preparations above the double line are intended for use as mineralocorticoids.

[1] TA = topical application to skin or mucous membranes in creams, solutions, ointments, gels, lotions, or aerosols. O = ophthalmic solution, suspension, or ointment. I = nasal or oral inhalation.

[2] *See* Figure 63–3 for structure.

[3] *See* Table 63–3 for chemical name.

[4] Beclomethasone, 9α-chloro,16β-methylprednisolone,17,21-dipropionate; amcinonide, 9α-fluoro,16α-hydroxyprednisolone, cyclic 16,17-acetal with cyclic pentanone,21-acetate; clocortolone, $\Delta^{1,2}$,6α-fluoro,9α-chloro,16α-methylcorticosterone 21-pivalate; desonide, 16α-hydroxyprednisolone, cyclic 16,17-acetal with acetone; desoximetasone, $\Delta^{1,2}$,9α-fluoro,16α-methylcorticosterone; diflorasone diacetate, 6α,9α-difluoro,16β-methylprednisolone, 17,21-diacetate; flumethasone, 6α,9α-difluoro,16α-methylprednisolone; fluocinolone, 6α,9α-difluoro, 16α-hydroxyprednisolone,16,17-acetal with acetone; fluocinonide, 6α,9α-difluoro,16α-hydroxyprednisolone,16,17-acetal with acetone,21-acetate; fluorometholone, $\Delta^{1,2}$,9α-fluoro,6α-methyl, 11β,17-dihydroxyprogesterone; flurandrenolide, 6α-fluoro,16α-hydroxycortisol,16,17-acetal with acetone; halcinonide, 21-chloro,9α-fluoro,11β,16α,17-trihydroxypregn-4-ene-3,20-dione,16,17-acetal with acetone; medrysone, 11β-hydroxy,6α-methylprogesterone.

The use of corticosteroids for days or a few weeks does not lead to adrenal insufficiency upon cessation of treatment, but prolonged therapy with corticosteroids may result in suppression of pituitary-adrenal function that can be slow in returning to normal. Graber and coworkers (1965) found that the processes of recovery of normal pituitary and adrenal function required 9 months in some patients. During this recovery period and for an additional 1 to 2 years, the patient may need to be protected during stressful situations, such as surgery or severe infections, by the administration of corticosteroids. Dixon and Christy (1980) have discussed the complex clinical problems that can be provoked by withdrawal from steroid therapy.

In addition to pituitary-adrenal suppression, the principal complications resulting from prolonged therapy with corticosteroids are fluid and electrolyte disturbances; hyperglycemia and glycosuria; increased susceptibility to infections, including tuberculosis; peptic ulcers, which may bleed or perforate; osteoporosis; a characteristic myopathy; behavioral disturbances; posterior subcapsular cataracts; arrest of growth; and Cushing's habitus, consisting in "moon face," "buffalo hump," enlargement of supraclavicular fat pads, "central obesity," striae, ecchymoses, acne, and hirsutism.

Hypokalemic alkalosis and *edema* are infrequently encountered in patients who are treated with synthetic corticosteroid congeners and almost never in patients taking the 16-substituted compounds. *Glycosuria* can usually be managed with diet and/or insulin, and its occurrence should not be an important factor in the decision to continue corticosteroid therapy or to initiate it in diabetic patients.

Increased susceptibility to infection in patients treated with corticosteroids is not specific for any particular bacterial or fungal pathogen. If infection develops in a patient treated with corticosteroids, the dose may be maintained or increased and the best available treatment for the infection vigorously administered. Corticosteroid therapy may be initiated in patients having known infections of some consequence if effective, specific chemotherapy can be administered concomitantly with the hormones. However, in these circumstances the physician should be confident that the corticosteroid is needed, that the pathogen has been identified, and that chemotherapy will be effective.

Peptic ulceration is an occasional complication of corticosteroid therapy. The high incidence of hemorrhage and perforation in these ulcers and the insidious nature of their development make them severe therapeutic problems. However, there has

been disagreement about the incidence of these ulcers, and some studies have concluded that evidence does not support an association between peptic ulcers and treatment with glucocorticoids (Conn and Blitzer, 1976). It is also not known whether there is an interaction between glucocorticoids and nonsteroidal anti-inflammatory drugs, such as aspirin, which, by themselves, can cause ulcers. In a recent survey of the literature, Messer and associates (1983) concluded that steroid therapy approximately doubles the risk of ulcer (*see also* Spiro, 1983).

Myopathy, characterized by weakness of the proximal musculature of arms and legs and of their associated shoulder and pelvic muscles, is occasionally seen in patients taking large doses of corticosteroids. It may occur soon after treatment is begun and be sufficiently severe to prevent ambulation. It is not specific for synthetic corticosteroid congeners, for it is found in endogenous Cushing's syndrome. It is a serious complication and an indication for withdrawal of therapy. Recovery may be slow and incomplete (*see* Mandel, 1982).

Behavioral disturbances may take various forms, for example, nervousness, insomnia, changes in mood or psyche, and psychopathies of the manic-depressive or schizophrenic type. Suicidal tendencies are not uncommon. It is no longer believed that previous psychiatric problems predispose to behavioral disturbances during therapy with glucocorticoids. Conversely, the absence of a history of psychiatric illness is no guarantee against the occurrence of psychosis during hormonal therapy.

Posterior subcapsular cataracts have been reported in children receiving corticosteroid therapy. Many patients with rheumatoid arthritis who receive 20 mg of prednisone per day for 4 years develop cataracts (Levine and Leopold, 1973); it is possible that patients with this disease are particularly susceptible to this complication. The problem of corticosteroid-induced cataracts has been reviewed by Lubkin (1977).

Osteoporosis and *vertebral compression fractures* are frequent serious complications of corticosteroid therapy in patients of all ages. Ribs and vertebrae, bones with a high degree of trabecular structure, are generally the most severely affected. Glucocorticoids appear to inhibit the activities of osteoblasts directly, and, because of their inhibition of calcium absorption by the intestine, glucocorticoids cause an increased secretion of parathyroid hormone (PTH). PTH stimulates the activity of osteoclasts (Hahn, 1978); thus, there is both decreased formation and increased resorption of bone. As noted above, corticosteroids also increase calcium excretion by the kidney. Osteoporosis is an indication for withdrawal of therapy and should be looked for regularly in radiographs of the spine in patients taking glucocorticoids for longer than a few months. Unfortunately, significant loss of bone must occur before it is apparent from radiography. The possibility of development of osteoporosis should be an important consideration in initiating and managing corticosteroid therapy, especially in postmenopausal women (*see* Baylink, 1983).

Inhibition or arrest of growth can result from the administration of relatively small doses of glucocorticoids to children. This cannot be overcome with exogenous human growth hormone (Morris *et al.*, 1968). The widespread inhibitory effect of the glucocorticoids on DNA synthesis and cell division discussed above is apparently responsible. Inhibition of growth by glucocorticoids has been reviewed by Loeb (1976).

THERAPEUTIC USES

With the exception of substitution therapy in deficiency states, the use of corticosteroids and their congeners in disease is largely empirical. From the experience accumulated since the introduction of glucocorticoids for clinical use, at least six therapeutic principles may be abstracted, as follows: (1) for any disease, in any patient, the appropriate dose to achieve a given therapeutic effect must be determined by trial and error and must be reevaluated from time to time as the stage and the activity of the disease alter; (2) a *single* dose of corticosteroid, even a large one, is virtually without harmful effects; (3) a few days of corticosteroid therapy, in the absence of specific contraindications, is unlikely to produce harmful results except at the most extreme dosages; (4) as corticosteroid ther-

apy is prolonged over periods of weeks or months, and to the extent that the dose exceeds the equivalent of substitution therapy, the incidence of disabling and potentially lethal effects increases; (5) except in adrenal insufficiency, the administration of corticosteroids is neither etiological nor curative therapy but only palliative by virtue of the anti-inflammatory effects; and (6) abrupt cessation of prolonged, high-dosage corticosteroid therapy is associated with a significant risk of adrenal insufficiency of sufficient severity to be threatening to life.

Translated into the terms of clinical practice, these general principles are equivalent to the following rules. When corticosteroids are to be administered over long periods, the dose must be the smallest one that will achieve a desired effect. This dose must be found by trial and error. Where the goal of therapy is relief of painful or distressing symptoms not associated with an immediately life-threatening disease, for example, rheumatoid arthritis, the initial dose should be small and gradually increased until pain or distress has been reduced to tolerable levels. Complete relief is not sought. At frequent intervals the dose should be gradually reduced until the development of more severe symptoms signals that the minimal acceptable dose has been found. When therapy is directed at an immediately life-threatening state, for example, pemphigus, the initial dose should be a large one, estimated to achieve, almost with certainty, control of the crisis. If some benefit is not observed in a short time, the dose should be doubled or tripled. When potentially lethal disease is controlled by large amounts of corticosteroid, reduction of the dose should be carried out under conditions that permit frequent, accurate observations of the patient. Under these circumstances it is essential to assess constantly the relative dangers of therapy and of the disease being treated.

The apparently innocuous character of a single administration of corticosteroid in amounts within the conventional therapeutic range justifies its use without a definite diagnosis for crises in which there exists some probability that life is threatened by primary adrenal or pituitary insufficiency. If one of these conditions is present, a single intravenous injection of a soluble corticosteroid may prevent immediate death and allow time for diagnostic procedures.

Short courses of systemic corticosteroids in large doses may properly be given for diseases that do not threaten life, in the absence of specific contraindications. The general rule is that long courses of therapy at high dosage should be reserved for life-threatening disease. On occasion, and for definite cause, when the patient is threatened with permanent disability, this rule is justifiably violated.

It is not possible to define the precise dose of glucocorticoids that will produce pituitary and adrenocortical suppression in a given patient, since there is considerable variation. In general, the higher the dose and the more prolonged the therapy, the greater is the likelihood of suppression.

Harter and associates (1963) suggested that some dissociation of therapeutic effects from certain undesirable metabolic effects can be achieved by the administration of a single large dose of corticosteroid every other day, in contrast to the usual daily multiple-dose schedule. A single dose every other day or at even longer intervals is acceptable therapy for some, but not all, patients with a variety of diseases modified by corticosteroid therapy. When this therapeutic regimen is possible, the degree of suppression of the pituitary and adrenal cortex can be minimized. Steroids that are long acting are not suitable for use by this dosage schedule.

Substitution Therapy. Insufficiency of secretion of the adrenal cortex results from structural or functional lesions of the adrenal cortex itself or from structural or functional lesions of the anterior pituitary. In either case, the patient may present with acute, catastrophic adrenal insufficiency (adrenal crisis) or chronic adrenal insufficiency. When the adrenal itself is the site of the lesion, all elements of normal adrenal secretion may be reduced or absent or the deficiency may be selective for one or more components of secretion.

Acute Adrenal Insufficiency. This disease is characterized by gastrointestinal symptoms, dehydration, weakness, lethargy, and hypotension. It is usually associated with disorders of the adrenal rather than the pituitary, although exceptions occur. It frequently follows abrupt withdrawal of high doses of corticosteroids.

The immediate needs of such patients are water, sodium, chloride, glucose, cortisol, and appropriate therapy for precipitating causes, for example,

infection, trauma, or hemorrhage. Inasmuch as these patients have a diminished capacity for a water diuresis and have often undergone some degree of cellular hydration, they are susceptible to water intoxication. The principal intravenous fluid should be isotonic sodium chloride solution. Glucose is required for nutrition and to prevent or treat hypoglycemia, but it should be given intravenously in isotonic sodium chloride solution. The total amount of intravenous fluid administered during the first 24 hours should not, in most instances, exceed 5% of ideal body weight. The patient should be monitored for evidence of rising venous pressure and pulmonary edema, because the functional capacity of the cardiovascular system is reduced by adrenocortical insufficiency. Cortisol (hydrocortisone) sodium succinate or cortisol sodium phosphate must be given in the intravenous fluids at a rate of 100 mg every 8 hours, following an initial intravenous injection of 100 mg. This provides a quantity of cortisol that is equal to the maximal daily rate of secretion in response to stress. In the period of transition from intravenous fluid therapy to normal diet and activity, intramuscular cortisol sodium succinate or sodium phosphate may be used in a dose of 25 mg every 6 or 8 hours.

For the treatment of suspected but unconfirmed acute adrenal insufficiency, 4 mg of dexamethasone sodium phosphate should be substituted for cortisol. In addition, ACTH (5 units per hour) should be given. Concentrations of cortisol and aldosterone in plasma and 17-hydroxycorticosteroids in urine are determined at the outset and at intervals during the course of treatment. A failure to obtain a response to ACTH (stimulation of steroid secretion) is diagnostic of adrenal insufficiency. A lack of response in terms of aldosterone indicates failure of the zona glomerulosa.

Chronic Adrenal Insufficiency. This disease results from adrenal surgery or destructive lesions of the adrenal cortex. It requires the administration of cortisol, 20 to 30 mg per day in divided doses. A common dose schedule is 20 mg on arising and 10 mg in the late afternoon. The circadian pattern of ACTH concentrations should be measured. If it rises above normal, multiple doses of cortisol may be required. Most patients will also require a potent mineralocorticoid. The most convenient drug to use for this purpose is fludrocortisone acetate. The usual adult dose is 0.1 to 0.2 mg daily. Some patients do not need a mineralocorticoid and are adequately treated with cortisone and generous dietary salt. Therapy is guided by the patient's sense of well-being, alertness, appetite, weight, muscular strength, pigmentation, blood pressure, and freedom from orthostatic hypotension.

Congenital Adrenal Hyperplasia. This is a familial disorder in which activity of one of several enzymes required for biosynthesis of corticosteroids is deficient. With diminished or absent production of cortisol, aldosterone, or both, and consequent lack of inhibitory feedback, the adrenal cortex is stimulated to the overproduction of other hormonally active steroids. The clinical presentation, laboratory findings, and treatment depend on which of the six enzyme deficiencies thus far described is responsible. Only the syndrome of 21-hydroxylase deficiency will be described here.

In about 90% of the patients with congenital adrenal hyperplasia there is a deficiency of 21-hydroxylase activity. When the deficiency is only partial, the usual case, cortisol is secreted at normal rates as a result of continuous hypersecretion of ACTH, with consequent overproduction of adrenal androgens and their precursors. Aldosterone secretion is approximately normal. Female children undergo virilization, female "pseudohermaphroditism," and male children show precocious development of secondary sex characteristics, "macrogenitosomia." Linear growth is accelerated in childhood, but the height at maturity is reduced by premature closure of the epiphyses.

In about 30% of patients with 21-hydroxylase deficiency, the enzymatic defect is sufficiently severe to compromise increased aldosterone secretion in response to a hypovolemic stimulus. Such patients are unable to conserve sodium normally, in addition to manifesting androgenic effects (Bongiovanni *et al.*, 1967).

All patients with congenital adrenal hyperplasia resulting from a 21-hydroxylase deficiency require substitution therapy with cortisol or a suitable congener, and those with a salt-losing tendency require, in addition, a sodium-retaining steroid. The usual oral dose of cortisol is about 0.6 mg/kg daily in four divided doses, the last one being given as late as possible in order to maintain pituitary suppression overnight. When parenteral substitution therapy is necessary, cortisone acetate may be given intramuscularly every other day. The mineralocorticoid usually given is fludrocortisone acetate, 0.05 to 0.2 mg per day. Therapy is guided by gain in weight and height, by excretion of urinary 17-ketosteroids, and by blood pressure. Sudden spurts of linear growth may indicate inadequate pituitary suppression and excessive androgen secretion, whereas growth failure suggests overtreatment.

A number of rare forms of congenital adrenal hyperplasia are known in which enzyme deficiencies of the adrenal cortex, with similar defects of the gonads, result in clinical and laboratory findings very different from those described above for 21-hydroxylase deficiency. The types described thus far are: "desmolase" deficiency (Camacho *et al.*, 1968), 3β-hydroxysteroid dehydrogenase deficiency (Bongiovanni *et al.*, 1967), 17α-hydroxylase deficiency (Goldsmith *et al.*, 1968), 11β-hydroxylase deficiency (Bongiovanni *et al.*, 1967), and 18-hydroxylase deficiency (David *et al.*, 1968). The clinical and laboratory findings and the treatment in these rare forms are quite different from those in 21-hydroxylase deficiency. The publications cited should be consulted for details.

Adrenal Insufficiency Secondary to Anterior Pituitary Insufficiency. This condition is not usually associated with the dramatic signs and symptoms characteristic of adrenal insufficiency resulting from disease of the adrenal cortex unless there are complicating circumstances, for example, unusual fluid losses, trauma, or starvation. Hypoglycemia is the most frequent cause of symptoms. Quantita-

tion of the electrolytes in plasma often reveals a dilutional hyponatremia. The administration of 20 mg of cortisol on arising and 10 mg in late afternoon is adequate replacement therapy for most patients with anterior pituitary insufficiency. This schedule mimics, to some extent, the normal diurnal cycle of adrenal secretion. Occasional patients require additional doses. When initiating treatment, it is customary to begin cortisol first and to add thyroid replacement therapy after adrenal insufficiency is under some degree of control, on the grounds that the administration of thyroid to a hypopituitary patient may precipitate acute adrenal insufficiency. Additional treatment is necessary during periods of stress. Cortisol, 300 to 400 mg per day, should be given to approximate the normal response to severe stress.

Therapeutic Uses in Nonendocrine Diseases. Brief outlines of important uses of corticosteroids in diseases other than those involving the pituitary-adrenal complex are set forth below. The disorders discussed are not inclusive, but rather a representative list of the more common diseases for which the glucocorticoids are used.

The dosage of glucocorticoids varies greatly with the condition being treated. In the following discussion approximate doses of a representative corticosteroid congener, usually prednisone, are suggested. It is not meant to imply that prednisone has peculiar merit in general or for any particular disease over the other congeners. For comparison of doses of glucocorticoids, *see* Table 63–3.

Arthritis. In *rheumatoid arthritis*, the criterion for initiating corticosteroid therapy is progressive disease with consequent disability, despite intensive treatment with rest, physical therapy, aspirin-like drugs, gold, and other agents. The decision to embark upon a program of hormone therapy must be made with due consideration for the fact that corticosteroid therapy, once started, may have to be continued for many years or for life, with the attendant risks of serious complications. The initial dose should be small and increased slowly until the desired degree of control is attained. The symptomatic effect of small reductions should be frequently tested in order to maintain the dose as low as possible. Complete relief is not sought. A regimen of rest, physical therapy, and aspirin-like drugs is continued. The usual initial dose is about 10 mg of prednisone (or equivalent) per day in divided doses. Optimal therapy for some patients with painful symptoms confined to one or a few joints may be intra-articular injection of the steroid into the affected joints. Typical doses are 5 to 20 mg of triamcinolone acetonide or the equivalent, depending upon the size of the joint cavity.

In *osteoarthritis*, intra-articular injection of corticosteroids is recommended for treatment of episodic manifestations of acute inflammation: local heat, swelling, and pain. Injections for this purpose should be infrequent because, in both rheumatoid arthritis and osteoarthritis, a significant incidence of painless destruction of the joint, reminiscent of Charcot's arthropathy, may be associated with repeated intra-articular injections of corticosteroids.

Rheumatic Carditis. Corticosteroids are reserved for patients failing to respond to salicylates and as initial therapy for patients severely ill with fever, acute congestive heart failure, arrhythmia, and pericarditis; acute manifestations are more rapidly suppressed by corticosteroids than by salicylates, a possibly lifesaving difference in a moribund patient. A dose of approximately 40 mg of prednisone or equivalent is usually given daily, in divided amounts, although much larger doses may on occasion be required. Reactivation of the disease occurs in a number of instances following withdrawal of steroid therapy. For this reason it has been suggested that salicylates be given concurrently with corticosteroids and be continued through and after the period of withdrawal of hormone therapy.

Renal Diseases. Corticosteroids do not modify the course of acute or chronic glomerulonephritis. However, patients with some forms of the *nephrotic syndrome* attributable to systemic lupus erythematosus or to primary renal disease, except renal amyloidosis, may be benefited by corticosteroid therapy. A typical therapeutic regimen consists in the daily administration, in divided doses, of 60 mg of prednisone or equivalent (2 mg/kg of edema-free body weight in children) for 3 or 4 weeks. If a remission with a diuresis and decreased proteinuria occurs during this period, maintenance treatment is continued for as long as a year. For this, the daily dose of prednisone is given only for the first 3 days of each week (Bacon and Spencer, 1973).

Collagen Diseases. The manifestations of most of the diseases in this group are controlled by glucocorticoids. An exception is *scleroderma*, which is generally considered refractory to these agents. It is important to distinguish between scleroderma and *mixed connective tissue disease syndrome*, which is responsive to steroids (Yount *et al.*, 1973). *Polymyositis, polyarteritis nodosa*, and the granulomatous-polyarteritis group (*Wegener's granulomatosis, temporal-cranial arteritis*, and *polymyalgia rheumatica*) are treated with daily doses of prednisone, approximately 1 mg/kg or equivalent, to induce a remission. The dose is then tapered down to the minimally effective level. Glucocorticoids decrease morbidity in all these diseases and prolong the survival times of patients with polyarteritis nodosa and Wegener's granulomatosis. *In temporal (giant-cell) arteritis, adequate steroid therapy is necessary to prevent the blindness that occurs in about 20% of untreated cases. Fulminating systemic lupus erythematosus* is a life-threatening condition, the manifestations of which should be suppressed by adrenocorticosteroid therapy with doses large enough to produce a prompt effect. Treatment usually consists in a 1-mg/kg daily dose of prednisone or equivalent. Within 48 hours a reduction of fever and improvement in the signs and symptoms of arthritis, pleuritis, or pericarditis should be observed. If not, the dose should be increased in 20-mg increments daily until a favorable response occurs. After the acute episode has been brought under control, corticosteroid therapy should be reduced by small steps, for example, 5 mg of prednisone per week, until signs or symp-

toms warn against further reductions. Salicylate or related drugs are then introduced and may permit a further reduction of corticosteroid dosage (Robinson, 1962). The treatment of systemic lupus erythematosus with a combination of glucocorticoids and antimetabolites, such as azathioprine, or the alkylating agent cyclophosphamide, is still experimental and not recommended for general use (Deker, 1982).

Allergic Diseases. The manifestations of allergic disease that are of limited duration, such as *hay fever, serum sickness, urticaria, contact dermatitis, drug reactions, bee stings,* and *angioneurotic edema,* can, if necessary, be suppressed by adequate doses of glucocorticoids given as a supplement to the primary therapy. It must be emphasized, however, that the effects of the steroids require some time to develop, and *severe reactions such as anaphylaxis and angioneurotic edema of the glottis require immediate therapy with epinephrine, 0.5 to 1.0 ml of a 1:1000 solution (0.5 to 1.0 mg) subcutaneously.* In life-threatening situations steroids may be given intravenously; dexamethasone sodium phosphate (8 to 12 mg or equivalent) is appropriate. In less severe diseases, such as serum sickness or hay fever, antihistaminic compounds are the drugs of first choice.

Bronchial Asthma. The corticosteroids should *not* be used routinely in the treatment of any asthmatic condition, acute or chronic, that can promptly be brought under moderate control with other measures. In *status asthmaticus,* cortisol sodium succinate (50 to 100 mg) is administered by intravenous infusion over 8 hours. The procedure is repeated daily until the acute attack is under control, following which the patient is given 10 mg of prednisone twice daily for 4 or 5 days. The dose is then reduced in steps and withdrawal planned for about the tenth day after initiation of the prednisone therapy. Under favorable circumstances, the patient can subsequently be managed once again with his prior medication.

In the treatment of *severe chronic bronchial asthma,* or *chronic obstructive pulmonary disease,* uncontrolled by other measures, the administration of a corticosteroid may be considered. The decision must be made with great care since the majority of patients, once started on corticosteroid therapy, remain indefinitely on such therapy. While some patients are effectively managed with inhalation of beclomethasone dipropionate, oral administration of prednisone in daily doses of 5 to 10 mg is required more frequently. Patients who have been taking a glucocorticoid orally must continue this medication in slowly decreasing dosage when inhalation therapy with beclomethasone is begun. Asymptomatic oropharyngeal candidiasis develops in a high percentage of patients using beclomethasone (Webb-Johnson and Andrews, 1977).

Ocular Diseases. Corticosteroids are frequently used to suppress inflammation in the eye, and employed properly they are often responsible for preservation of sight. Levine and Leopold (1973) list 28 disorders of the eye that respond to corticosteroids. They are administered locally for disease of the outer eye and anterior segment. Both natural and synthetic corticosteroids attain therapeutic concentrations in the aqueous humor following instillation into the conjunctival cul-de-sac. For disease of the posterior segment, systemic administration is required.

A typical prescription is 0.1% dexamethasone phosphate solution (ophthalmic), 2 drops in the conjunctival sac every 4 hours while awake, and 0.05% dexamethasone phosphate ointment (ophthalmic) at bedtime. For inflammations of the posterior segment of the eye, usual daily doses are approximately 30 mg of prednisone or equivalent, administered orally in divided doses.

It has been convincingly demonstrated that topical corticosteroid therapy *frequently induces intraocular hypertension* in normal eyes and further increases pressure in eyes with initially elevated pressure. The glaucoma has not always been reversible on cessation of corticosteroid treatment. It has been recommended that intraocular pressure be monitored when corticosteroids are applied to the eye for more than 2 weeks.

The local administration of corticosteroids to patients with bacterial, viral, or fungal conjunctivitis *may mask evidences of progression of the infection until sight is lost.* Corticosteroids are *contraindicated in herpes simplex* (dendritic keratitis) of the eye, because progression of the disease and irreversible clouding of the cornea may occur. Topical steroids should not be used in the treatment of mechanical lacerations and abrasions of the eye. They delay healing and promote the development and spread of infection.

Skin Diseases. The development of corticosteroid preparations suitable for topical administration has revolutionized the therapy of the more common varieties of skin disease. Maibach and Stoughton (1973) have divided 20 dermatological disorders that respond to topical corticosteroids into those that are very responsive and those that require higher concentrations of steroids, occlusion of the drug under a plastic film, or intralesional administration. Attention must be paid to the concentration of steroid used, and there are a large number of preparations of various concentrations available for topical use (Table 63–4). A typical prescription for an eczematous eruption is 1% cortisol ointment applied locally twice daily. Effectiveness is enhanced by application of the cream or ointment under a transparent plastic wrapping. Unfortunately, systemic absorption is also enhanced, occasionally sufficiently to suppress the pituitary-adrenal axis or to produce Cushing's syndrome. Adrenocorticosteroids are administered systemically for severe episodes of acute skin disorders and exacerbations of chronic disorders. The dose is usually 40 mg per day of prednisone or equivalent. Systemically administered corticosteroids may be lifesaving in *pemphigus.* Up to 120 mg of prednisone or equivalent per day may be required to control the disease.

Diseases of the Intestinal Tract. Patients severely ill with untreated *celiac sprue* can often benefit from a course of glucocorticoid therapy given at the same time that management with a gluten-free diet is begun. Prednisolone, 30 mg per day or

equivalent, is continued for 3 to 4 weeks. Patients who fail to respond to a gluten-free diet are helped by lower doses of prednisolone (7 to 12 mg per day or equivalent) for an indefinite period (Wall, 1973).

Corticosteroid therapy is indicated in selected patients with *chronic ulcerative colitis*. Mildly ill patients with bowel symptoms but without disabling systemic symptoms usually can and should be managed with rest, diet, sedation, anticholinergic agents, and chemotherapy. However, patients who do not improve should have a trial of methylprednisolone acetate, 40 mg or equivalent, in a nightly retention enema, in an attempt to induce remission. Alternate-day therapy may be effective. Severely ill patients with fever, anorexia, anemia, and malnutrition often improve dramatically when given systemic corticosteroid therapy. Large doses, 60 to 120 mg per day of prednisone, or the equivalent, are recommended. Major complications of ulcerative colitis may occur despite corticosteroid therapy. Signs and symptoms of intestinal perforation and peritonitis may be difficult to detect during corticosteroid treatment (ReMine and McIlrath, 1980).

Cerebral Edema. Corticosteroids are of value in the reduction or prevention of cerebral edema associated with neoplasms, especially those that are metastatic. In spite of widespread use of glucocorticoids for treatment of the cerebral edema due to trauma or cerebrovascular accidents, there is no convincing evidence of their value in these conditions (Nelson and Dick, 1975).

Malignancies. The chemotherapy of *acute lymphocytic leukemia* and *lymphomas* has been greatly improved by the introduction of therapy with multiple agents, and glucocorticoids are used because of their antilymphocytic effects. At the present time these diseases are treated in a complex fashion with rigidly scheduled sequences of combined drug therapy. Prednisone is commonly used in conjunction with an alkylating agent such as cyclophosphamide, an antimetabolite, and a vinca alkaloid (*see* Chapter 55).

Objective tumor regression in *carcinoma of the breast* can be induced by glucocorticoids in about 15% of patients; prednisolone (30 mg per day) has been the usual treatment. The presumed mechanism by which the corticosteroids act in these patients is through adrenocortical suppression, with an accompanying decrease in production of androgens, which are precursors of tumor-stimulating estrogens (Brennan, 1973). A beneficial response should be expected only when the tumor has estrogen and/or progesterone receptors. Other forms of therapy are usually more effective.

Diseases of the Liver. The use of glucocorticoids in the treatment of hepatic diseases has been the subject of controversy. Careful studies have now indicated several diseases of the liver in which therapy with steroids significantly improves survival rates; these are *subacute hepatic necrosis* and *chronic active hepatitis, alcoholic hepatitis,* and *nonalcoholic cirrhosis in females* (Lesesne and Fallon, 1973; Copenhagen Study Group for Liver Diseases, 1974). Only certain patients with chronic active hepatitis should receive steroid therapy. Those who benefit have symptomatic disease, histological evidence of severe disease, and a negative reaction for hepatitis B surface antigen (Berk *et al.*, 1976). Treatment of *subacute hepatic necrosis* and *chronic active hepatitis* includes prednisolone, 60 to 100 mg per day; the dose is tapered as the disease improves. Treatment of *alcoholic hepatitis* with corticosteroids is reserved for patients who are severely ill, with evidence of hepatic encephalopathy. Prednisone (40 mg per day) is given for 1 month, followed by withdrawal over a period of 2 to 4 weeks. *Nonalcoholic cirrhosis in women* should be treated with glucocorticoids if the patient does not have ascites. Daily dosages average 15 to 20 mg of prednisone or equivalent when they are adjusted to the needs of the individual patients. The data indicate that *steroid treatment lowers survival rates when ascites is present.* Treatment of cirrhotic male patients with steroids has not been shown to be beneficial.

Shock. While corticosteroids are often administered to patients in shock, there is no convincing evidence to indicate that such therapy is efficacious.

Miscellaneous Diseases. Sarcoidosis is treated with prednisone, approximately 1 mg/kg per day or equivalent, to induce a remission. Maintenance doses, which are often required for long periods of time, may be 10 mg of prednisone per day or less. In this, as in other diseases treated by prolonged steroid therapy, patients with positive tuberculin reactions or other evidence of tuberculosis should receive prophylactic antituberculosis therapy. In *thrombocytopenia,* prednisone, 0.5 mg/kg or equivalent, is used to decrease the bleeding tendency. In severe cases and for initiation of treatment of *idiopathic thrombocytopenia,* daily doses of prednisone, 1 to 1.5 mg/kg, are employed. *Hemolytic anemias* with a positive Coombs' test are treated with prednisone, 1 mg/kg per day or equivalent. If hemolysis is severe, therapy is initiated with 100 mg of cortisol intravenously; as the disease improves, the dose is decreased. Small maintenance doses may be needed for several months if a positive response is obtained. In *organ transplantation,* high doses of prednisone (50 to 100 mg) are given at the time of the transplant surgery, usually in conjunction with immunosuppressive agents. Smaller maintenance doses (10 to 20 mg per day) are continued indefinitely, and the dosage is increased if rejection is threatened. In *aspiration of gastric contents,* prednisone (50 to 100 mg) is given for 2 to 3 days to suppress the inflammatory reaction in the lung and to prevent development of pulmonary abscess. However, corticosteroids are probably effective only if they are administered within several hours of the aspiration.

DIAGNOSTIC APPLICATIONS OF ADRENOCORTICAL STEROIDS

Potent synthetic congeners of cortisol reduce urinary excretion of cortisol metabolites by inhibition of pituitary ACTH release. The dose required is

small, in gravimetric terms, that it contributes only negligibly to the urinary steroids. Liddle (1960) reported that the administration of 0.5 mg of dexamethasone every 6 hours for a total of eight doses results in a marked suppression of excretion of cortisol metabolites in normal persons, but does not suppress urinary steroids in individuals with Cushing's syndrome. This test is useful in distinguishing persons with some nonspecific elevation of steroid excretion, for example, that due to obesity or stress, from patients with Cushing's syndrome. The administration of 2 mg of dexamethasone every 6 hours for a total of eight doses usually causes a suppression of cortisol secretion in patients with pituitary-dependent hypercorticism, but ordinarily has little if any effect on the urinary steroids of patients with adrenal neoplasms or ACTH-producing tumors (Meador *et al.*, 1962). However, "suppressible" tumors have been reported. The results of these tests are likely to be most definite if the urinary steroids are measured daily for 2 days before and for at least 2 days during administration of the suppressing agent. Variations of this procedure (shorter test period and measurement of plasma cortisol rather than urinary metabolites) have been described (Sawin *et al.*, 1968).

INHIBITORS OF THE BIOSYNTHESIS OF ADRENOCORTICAL STEROIDS

Three pharmacological agents have proven most useful as inhibitors of adrenocortical secretion. *Mitotane* (*o,p'*-DDD), an adrenocorticolytic agent, is discussed in Chapter 55. *Metyrapone* and *aminoglutethimide* are discussed here. The subject has been reviewed by Temple and Liddle (1970).

Metyrapone. Metyrapone reduces cortisol production by inhibition of the 11β-hydroxylation reaction. Metyrapone also inhibits side chain cleavage to some degree (Cheng *et al.*, 1974), but this block is largely overcome when ACTH stimulates the gland. The biosynthetic process is terminated at 11-desoxycortisol (Figure 63–3), a compound that has practically no inhibitory influence on the secretion of ACTH. In the normal person, a compensatory increase in ACTH secretion follows, and the secretion of 11-desoxycortisol, a "17-hydroxycorticoid," is markedly accelerated. Consequently, in normal persons, administration of metyrapone induces increased plasma ACTH and renal excretion of "17-hydroxycorticoids."

Metyrapone is used to test the capacity of the pituitary to respond to a decreased concentration of plasma cortisol. A response that is greater than normal is usually found in patients with Cushing's syndrome of pituitary origin. In most cases of Cushing's syndrome due to ectopic production of ACTH there is no response to the drug. Administration of metyrapone to patients with disease of the hypothalamico-pituitary complex who are unable to achieve a compensatory increase in the rate of secretion of ACTH is, of course, not followed by increased renal excretion of 17-hydroxycorticoids.

The ability of the adrenal to respond to ACTH should be demonstrated before metyrapone is employed, for two reasons: (1) administration of metyrapone can be used as a test for normal hypothalamico-pituitary function only if the adrenal glands are capable of responding to ACTH, and (2) the drug may induce acute adrenal insufficiency in patients with reduced adrenal secretory capacity. Metyrapone also inhibits synthesis of aldosterone, which, like cortisol, is an 11β-hydroxylated compound. However, metyrapone does not typically cause a deficiency of mineralocorticoids, with a consequent loss of sodium and retention of potassium, because the inhibition of the 11β-hydroxylation reaction results in an increased production of 11-desoxycorticosterone, a mineralocorticoid.

Metyrapone has been used successfully to treat the hypercortisolism that results from adrenal neoplasms that function autonomously and from ectopic ACTH production by tumors. Its use in treatment of Cushing's syndrome resulting from hypersecretion of ACTH by the pituitary is controversial (Orth, 1978; Gold, 1979). Metyrapone has been used experimentally in patients with Cushing's syndrome during the period of time required for radiation treatment to become effective (Orth, 1978). Long-term treatment with metyrapone can cause hypertension as the result of excessive secretion of desoxycorticosterone.

Metyrapone (METOPIRONE) is 2-methyl-1,2-di-3-pyridyl-1-propanone. The drug is marketed as 250-mg oral tablets. Following two 24-hour control periods, the drug is given orally in the dose of 750 mg every 4 hours for six doses. Maximal urinary excretion of 11-desoxycorticosteroids is observed on the next day.

Aminoglutethimide. This compound, α-ethyl-*p*-aminophenyl-glutarimide, inhibits the conversion of cholesterol to 20α-hydroxycholesterol. This inhibition of the first reaction of steroidogenesis from cholesterol interrupts production of both cortisol and aldosterone.

Aminoglutethimide has been used successfully to decrease the hypersecretion of cortisol in autonomously functioning adrenal tumors and in hypersecretion resulting from ectopic production of ACTH. It has also been used in combination with metyrapone in the treatment of Cushing's syndrome that results from hypersecretion of ACTH by the pituitary (*see* Gold, 1979). Substitution of physiological doses of cortisol may be required to prevent adrenal insufficiency.

Aminoglutethimide (CYTADREN) is marketed as 250-mg oral tablets. The suggested dosage is 250 mg every 6 hours. The dose is increased by 250 mg per day at 1- or 2-week intervals until side effects prohibit further increments or until a daily dose of 2 g is achieved.

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Glucocorticoids

Disease Mechanism II: Inflammation

Powerful Anti-Inflammatory Compounds

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Drug Summary: Glucocorticoids are powerful anti-inflammatory compounds that have the ability to inhibit all stages of the **inflammatory response**. Common glucocorticoids include **prednisone**, **dexamethasone**, and **hydrocortisone**. While glucocorticoids are widely used as drugs to treat various inflammatory conditions, prolonged glucocorticoid use may have adverse side effects such as immunosuppression, fluid shifts, brain changes, and psychological changes. Physicians are therefore very cautious about prescribing these medications, especially for long periods of time.

What are glucocorticoids?

Natural glucocorticoids are steroid hormones with powerful anti-inflammatory effects produced by the human body. Glucocorticoid *drugs* are usually synthetic compounds that have anti-inflammatory effects similar to those of natural glucocorticoids.

Natural glucocorticoids are produced by the cortex of the **adrenal gland**. The adrenal glands are organs located immediately above our kidneys (ad = top of, renal = kidney). They are divided into two distinct regions:

- The **adrenal medulla** comprises the inner portion of the adrenal gland. It is the source of the body's stress hormones: **norepinephrine** (noradrenaline) and **epinephrine** (adrenaline).

- The **adrenal cortex** comprises the outer portion of the adrenal gland. It secretes several types of steroid hormones collectively known as **corticosteroids**. There are two main types of corticosteroids: **mineralcorticoids** and glucocorticoids. Mineralcorticoids like aldosterone are responsible for the maintenance of salt and fluid balance in the body. Glucocorticoids like **cortisol** and **cortisone** affect **metabolism** and inhibit inflammation.

Drugs with glucocorticoid activity are compounds that have similar effects to the natural steroid hormones produced by the adrenal cortex. While glucocorticoid drugs are steroids, they are unlike the **anabolic** steroids that some athletes take to build up and increase their muscle mass. Glucocorticoids are **catabolic** steroids, which means that they are designed to break down the body's stored resources through

their various metabolic effects. As stated above, glucocorticoids have two principal effects in the body: metabolic and anti-inflammatory. It therefore follows that glucocorticoid drugs affect both metabolism and inflammation.

Metabolic Effects

The name "glucocorticoid" derives from early observations that these hormones were involved in **glucose** (sugar) metabolism. During times when no food is being taken into the body, glucocorticoids stimulate several processes that serve to increase and maintain normal glucose concentrations in the blood. These processes include:

- Stimulation of glucose production in cells, particularly in the liver.
- Stimulation of fat breakdown in adipose (fat) tissues.
- Inhibition of glucose and fat storage in cells.

Anti-Inflammatory Effects

Glucocorticoids (both naturally produced and synthetic) are powerful anti-inflammatory compounds due to their ability to inhibit all stages of the inflammatory response, from redness to wound healing to cell proliferation. (For more on the relationship between inflammation and HD, click here). They affect all types of inflammatory responses, regardless of the mode of injury or type of disease-causing substance.

Glucocorticoids are steroid hormones, which can cross the cell membrane. Most of their effects involve interactions with **intracellular receptors** (receptors inside the cell). They bind to these receptors, and the hormone-receptor complex then enters the nucleus and acts as a **transcription factor**.

Transcription is the set of processes in the nucleus by which the base sequence "code" of DNA is converted to a sequence of complementary bases in **mRNA**. (For more on DNA, complementary and nitrogenous bases, click here.) Once mRNA is formed from transcription, it is transported into the cytoplasm where it is used as to help in the construction of a **protein** molecule. The process by which the protein molecule is formed from the mRNA blueprint is called **translation**.

Glucocorticoids bind to **glucocorticoid receptors** (GR) inside the cell and form a **glucocorticoid-GR complex**. This complex enters the nucleus and causes changes that alter the synthesis of mRNA from the DNA molecule, thereby altering the production of different proteins. Glucocorticoids can cause an increase in the production of certain proteins and a decrease in the production of other proteins by binding to key sites in the gene and enhancing or suppressing their transcription into mRNA. Glucocorticoids have also been found to cause changes in the mRNA molecule itself. Modifications to the mRNA can further alter the production of proteins in the cell.

Studies have shown that glucocorticoids can suppress the production of proteins involved in inflammation (resulting in their role as anti-inflammatory compounds).

Aside from interfering with the transcription of enzymes involved in inflammation, glucocorticoids further suppress inflammation by activating a group of enzymes known as **lipocortins**. Lipocortins have been found to inhibit or slow the action of **phospholipase A2** (PLA2), a key enzyme involved in the release of **arachidonic acid** (AA) from the cell membrane.

Arachidonic acids are a type of omega-6 **fatty acid**. The omega-6 fatty acids in our body often come from the vegetable oils and animal meats in our food. Once arachidonic acid is in our body, it is usually incorporated into our cell **membranes**. When a cell is damaged or under attack by foreign substances, arachidonic acid is released from the cell membrane and is converted into substances such as **prostaglandins** which mediate inflammation. Free arachidonic acid is converted into inflammatory prostaglandins by enzymes known as COX-2. (For more information on COX-2 enzymes, click here.)

Release of arachidonic acids require the activation of the enzyme **PLA2**. As stated previously, lipocortins inhibit PLA2 activity. By activating lipocortins, glucocorticoids cause the inhibition of PLA2, thereby inhibiting release of **AA** and consequent prostaglandin synthesis in the cell. Because lower amounts of inflammatory prostaglandins are synthesized, inflammation is suppressed and damage caused by chronic inflammation is decreased.

Glucocorticoids can also directly inhibit COX-2 enzymes directly. More details on these studies can be found at the section Research on Glucocorticoids.

Problems with glucocorticoid drugs

We have discussed how glucocorticoids can have both metabolic and anti-inflammatory effects. So far, it has been impossible to give glucocorticoid treatments that have only anti-inflammatory effects. Glucocorticoids have been found to increase blood glucose levels as well as suppress calcium absorption through their various metabolic affects. As such, long-term anti-inflammatory therapy with glucocorticoids can often lead to swelling, skin changes, decreased immunity, and psychological changes. More severe side effects such as **diabetes** or **osteoporosis** can also occur (even short-term glucocorticoid therapy tends to cause the patient to become temporarily diabetic.) Moreover, patients on long-term glucocorticoid therapy must be gradually tapered off their medications when discontinuing them in order to avoid rebound effects produced by the body.

In addition to the difficulty of separating the metabolic and anti-inflammatory effects of glucocorticoids, most synthetic drugs often referred to as glucocorticoids are actually synthetic corticosteroids. These synthetic drugs have both mineralcorticoid and glucocorticoid activity. However, in a particular compound, one type of activity will predominate over the other. Synthesis of pure glucocorticoid drugs has so far been elusive.

Commonly prescribed steroid drugs:

- **Prednisone and Prednisolone** – Most commonly used glucocorticoid

because of its high glucocorticoid activity. Prednisone is transformed by the liver into prednisolone. Prednisolone may be administered in tablet form or produced by the body from prednisone. These medications are often considered to be interchangeable.

- **Dexamethasone** – Has a particularly high glucocorticoid activity and low mineralcorticoid activity and can therefore be used in high doses. Often used to reduce nerve swelling following neurotrauma and neurosurgery.
- **Hydrocortisone** – Has much more mineralcorticoid activity than Prednisone and is therefore not suitable for long-term use internally. Externally, it is used extensively as a cream or lotion for skin conditions such as rashes or itches.

Research on Glucocorticoids

Newton, et al. (1998) conducted an experiment to try to explain the mechanism by which the glucocorticoid dexamethasone suppresses the production of mediators involved in inflammation. Previous studies indicate that synthetic drugs such as dexamethasone act by mimicking the natural glucocorticoid **cortisol** in binding to the glucocorticoid receptor (GR). The glucocorticoid-GR complex then moves to the nucleus, where it can activate transcription of anti-inflammatory genes.

This study investigated how glucocorticoids cause suppression of inflammatory mediators such as COX-2 and inflammatory prostaglandins, and proposed possible mechanisms to explain the suppressive effect of glucocorticoids and their inhibitory effects on various transcription factors.

Glucocorticoids have been found to interact with two transcription factors that help in the transcription of inflammatory genes. These factors, **NF-kappa B** and **AP-1** are believed to interact with the GR complex. Scientists believe that the both the NF-kappa B/GR and AP-1/GR interactions result in the decreased transcription of COX-2 mRNAs.

The researchers in this study also discovered that aside from its interactions with various transcription factors, dexamethasone is capable of suppressing COX-2 production by another mechanism as well. The researchers exposed cells to molecules that induce the production of COX-2 proteins and the release of inflammatory prostaglandins. To the surprise of the researchers, they discovered that dexamethasone not only lowers the rate of COX-2 mRNA transcription by about 44%, but it also causes structural changes in the COX-2 mRNA, further lowering the amount of COX-2 enzymes produced.

Previous experiments have shown that the COX-2 mRNA is extremely stable; its slow rate of degradation enables increased production of COX-2 proteins. Dexamethasone administration causes a decrease in the amount of COX-2 mRNA by suppressing its transcription and modifying the mRNA molecule. Modification of the COX-2 mRNA destabilized it, causing it to degrade at a faster rate, which in turn, decreases the production of COX-2 proteins.

The researchers are still uncertain as to what specific changes are induced by

dexamethasone to cause the modification found in the COX-2 mRNA. The results of this study indicate that glucocorticoids such as dexamethasone exert their anti-inflammatory effects through a variety of mechanisms: by interacting with transcription factors that slow COX-2 mRNA transcription and by modifying the COX-2 mRNA, destabilizing it, and increasing its rate of degradation.

The researchers proposed that the inflammation mechanisms of glucocorticoids need further study to determine how to control the production and activation of the various inflammatory mediators.

Aisen, et al. (2000) hypothesized that glucocorticoid administration may have beneficial effects for people with **Alzheimer's Disease** (AD). Their hypothesis was based on observations that the brains of people with AD showed increased inflammation. The researchers conducted a clinical trial to determine the usefulness of the glucocorticoid prednisone in slowing the rate of cognitive decline in people with AD.

The study enlisted 138 people with AD ages 50 or older. Half the participants were given a **placebo** and the other half were given prednisone. The treatment regimen consisted of an initial dose of 20 mg of prednisone daily for 4 weeks, lowered to a maintenance dose of 10 mg daily for one year, followed by a gradual tapering off of the drug for another 4 months.

Cognitive and behavioral assessments were done at specific intervals over the trial period to determine the efficacy of prednisone treatment. The researchers looked for changes over a one-year period as determined by the cognitive component of the **Alzheimer's Disease Assessment Scale** (ADAS) and other tests. Safety tests were also performed to monitor how the participants tolerated the drug.

Overall, the testing showed that low-dose prednisone did not slow the rate of cognitive decline when the prednisone-treated group was compared to those taking the placebo. Participants treated with prednisone also showed greater behavioral decline than those in the placebo group.

The researchers suggested some reasons why prednisone may not have been successful in treating AD. It is possible that the dosage given may not have been sufficient to suppress the destructive brain inflammatory activity. Much higher doses are used to treat inflammatory diseases of the brain such as **cerebral lupus**, a chronic autoimmune disease that causes inflammation in the brain. However, higher doses may not be safe for long-term treatment, particularly in the elderly. In this study, the incidence of **hyperglycemia** (greater than normal levels of blood glucose) and significant decline in bone density suggest that higher doses may cause substantial side effects.

Despite the negative results, the researchers believe that the study does not refute the potential benefit of anti-inflammatory compounds as treatment for neurological diseases such as **AD**. Rather, the study suggests that testing of other anti-inflammatory compounds such as **NSAIDs** (examples include **aspirin** and **ibuprofen**) or selective **COX-2 inhibitors** (examples include **rofecoxib** and

celecoxib) is critical in the search for the right combination of therapies for AD. (For more on NSAIDs and COX-2 inhibitors, click here) Both NSAIDs and COX-2 inhibitors have more limited anti-inflammatory effects in comparison to glucocorticoids and may be appropriate candidates for future trials. The study on prednisone serves as an important step in directing scientists toward what may or may not work at certain stages of AD, HD, and other neurological diseases that involve chronic inflammation.

Diamond, et al. (2000) investigated the role of glucocorticoid receptors (GR) in the aggregation of expanded polyglutamine proteins. Previous studies have shown that binding of the glucocorticoid dexamethasone to the GR receptor forms a glucocorticoid- GR complex that causes changes in the transcription of certain genes. The researchers in this study speculated that glucocorticoid may affect the transcription of certain proteins that could inhibit the aggregation of expanded polyglutamine proteins.

Studies indicate that several **polyglutamine diseases**, including HD, are caused by multiple C-A-G repeats within a unique gene. Other examples include **spinobulbar muscular atrophy** (SBMA), Huntington's Disease (HD), **dentatorubro-pallidoluysian atrophy**, and several **spinocerebellar ataxias** (SCAs) (For more on the polyglutamine diseases, click here.)

The altered genes result in the production of altered proteins that cause selective nerve cell death within the nervous system. For example, polyglutamine expansion within the **androgen receptor** (AR) protein results in SBMA, a disease associated with selective death of **motor nerve cells**. In the case of Huntington's disease, the **altered** huntington gene results in the production of an altered **huntingtin protein** that causes selective death of nerve cells found in the **basal ganglia**.

Studies indicate that the nerve cell death associated with these polyglutamine diseases may be linked to the formation of neuronal aggregates of the altered proteins. These altered proteins have been found to form aggregates called **neuronal inclusions** (NIs) in the nucleus of the nerve cell. (For more on NIs, click here.) Some studies have shown that reducing aggregate formation could improve conditions in animal models of the polyglutamine diseases.

The researchers in the current study attempted to discover ways on how these aggregations can be reduced. Based on the role of GRs as regulators of transcription, the researchers wondered whether they may have any role in the aggregation of polyglutamine proteins. The researchers found that the addition of dexamethasone to human kidney cells and mouse nerve cells expressing HD reduced the aggregation of the altered huntingtin protein. Similarly, dexamethasone administration to cells expressing SBMA showed decreased androgen receptor (AR) aggregation.

The results of the study indicate that aggregation of expanded polyglutamine proteins are regulated within the cell. The aggregation process can be manipulated through glucocorticoid-controlled gene expression. The researchers believe that the glucocorticoid-GR complex acts as a transcriptional regulator: in the nucleus, the complex binds to sites that can control and modulate the expression of nearby genes. It is possible that the transcriptional changes induced by the complex may

result in the production of proteins that could inhibit polyglutamine aggregation. What proteins are produced is still currently unknown.

More studies need to be done to identify the genes and proteins involved in the pathways that determine polyglutamine aggregations and nerve cell dysfunction. However, the results of this study raise the possibility that glucocorticoids could reduce polyglutamine aggregations. By reducing these aggregations, glucocorticoids could play essential roles in delaying or inhibiting the progression of diseases such as HD, SBMA, and possibly other polyglutamine diseases as well.

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-E. Tan, 6/15/02; Revised by P. Chang, 5/7/03

For further reading:

1. Newton, et al. "Repression of Cyclooxygenase-2 and Prostaglandin E2 Release by Dexamethasone Occurs by Transcriptional and Post-transcriptional Mechanisms Inolving Loss of Polyadenylated mRNA." Journal of Biological Chemistry. 1998; 273(48): 32312-32321.
   This study reports that the glucocorticoid dexamethasone acts to modify COX-2 mRNAs as well as regulate the transcription of some genes involved in inflammation.

2. Diamond, et al. "Regulation of expanded polyglutamine protein aggregation and nuclear localization by the glucocorticoid receptor." Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(2): 657-661.
   This study reports that glucocorticoids may reduce the aggregations found in polyglutamine diseases such as SBMA and HD.

3. Aisen, et al. "A randomized controlled trial of prednisone in Alzheimer's Disease." Neurology. 2000; 54:588.
   This study reports that prednisone was not effective in slowing the cognitive decline of people with AD.

4. Information on immunomodulation available online at:
   http://www.users.dircon.co.uk/~rosebud/drugs/Immune.html
   This page contains some information on the various pathways involved in the inflammatory response.

Click here to return to "Disease Mechanism II: Inflammation".
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PROSTAGLANDIN PRODUCTION BY MACROPHAGES AND THE EFFECT OF ANTI-INFLAMMATORY DRUGS

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1 Macrophages derived from peritoneal cavity inflammatory exudates of guinea-pigs produced substantial amounts of prostaglandin E_2-like activity during *in vitro* culture, so providing the basis for an experimental model of prostaglandin production during inflammatory reactions.

2 Dose-related inhibition of prostaglandin biosynthesis was demonstrated by 16 acidic non-steroidal anti-inflammatory drugs.

3 Seven anti-inflammatory glucocorticosteroid preparations inhibited prostaglandin production in a dose-related manner. The relative potencies of dexamethasone, prednisolone and hydrocortisone were consistent with clinical anti-inflammatory ranking. Cortisone, however, failed to inhibit macrophage prostaglandin production.

4 Three other agents used in the treatment of inflammatory joint diseases were examined. Sodium aurothiomalate inhibited prostaglandin production, although higher concentrations were toxic to macrophages. D-Penicillamine did not affect macrophage prostaglandin production. Colchicine, in contrast, enhanced prostaglandin production at some concentrations.

5 The probable significance of macrophages as a source of prostaglandins, during inflammatory responses, is discussed.

Introduction

There is considerable evidence that prostaglandins are involved as mediators of inflammation (Vane, 1972; Ferreira & Vane, 1974). However, the cellular origin of increased prostaglandin production in inflammatory reactions has remained uncertain and this aspect has received less attention than other criteria of mediator identification. The similar time course of appearance of prostaglandins, lysosomal enzymes and infiltrating leucocytes in carrageenin-induced exudates has been taken to suggest that the cellular infiltrate is the source of prostaglandin production (Anderson, Brocklehurst & Willis, 1971). The polymorphonuclear (PMN) leucocyte has been suggested as a possible source of inflammatory prostaglandins on the basis of observed *in vitro* prostaglandin E (PGE) production by rabbit PMN leucocytes (Higgs, McCall & Youlten, 1975). However, human peripheral blood PMN leucocytes showed little capacity for PGE production (Zurier & Sayadoff, 1975) and certainly no correlation exists between prostaglandin concentration and PMN leucocyte count in several types of inflammatory response (Levine, 1973; Blackham, Farmer, Radziwonik & Westwick, 1974; Glatt, Peskar & Brune, 1974).

The macrophage, another prominent infiltrating haematogenous cell, is known to contain a high proportion of arachidonic acid in membrane phospholipids (Mason, Stossel & Vaughan, 1972), to possess phospholipases A_1 and A_2, that can be activated by phagocytosis (Munder, Ferber, Modolell & Fischer, 1969; Franson & Waite, 1973) and to undergo lipid peroxidation following phagocytosis (Stossel, Mason & Smith, 1974). These observations would suggest that this cell type is a likely source of prostaglandins and we have previously reported that macrophages derived from inflammatory exudates produce substantial amounts of PGE-like activity during short-term *in vitro* culture (Bray, Gordon & Morley, 1974). The present experiments have examined the effects of a range of steroidal and non-steroidal anti-inflammatory drugs with established therapeutic value, on prostaglandin biosynthesis by macrophages, with the view to evaluating such an *in vitro* system for the screening of potential anti-inflammatory agents acting upon this component of the inflammatory response. A preliminary account of some of this work has been published (Bray & Gordon, 1976).

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Methods

Cell harvesting and culture

Peritoneal exudate cells were obtained from male, outbred Hartley guinea-pigs, weighing 300 to 500 g, following intraperitoneal injection of 20 ml 2% sterile starch solution (Sigma). The animals were killed 2 to 3 days later and the macrophage-rich cell populations were removed, through a ventral midline incision, by washing out the peritoneal cavity with 50 to 100 ml cold (4°C) Hanks balanced salt solution (Oxoid) containing penicillin (200 iu/ml) and streptomycin (100 iu/ml) (Glaxo). The cell pellet obtained after centrifugation at 400 *g* for 10 min was resuspended in 10 ml cold Hanks solution and the number of viable cells determined by vital dye exclusion (Eosin-Y: Oxoid) using a modified Neubauer haemocytometer (Hawksley). The cells were recentrifuged at 400 *g* for 10 min and suspended to a concentration of 1×10^6 viable cells/ml in cold Eagle's Minimal Essential Medium (MEM) (Flow Labs) containing penicillin (200 iu/ml) and streptomycin (100 iu/ml) supplemented with 10% heat-decomplemented foetal calf serum (Flow Labs).

Aliquots (0.5 ml) of the cell suspensions were dispensed into sterile, disposable, 12 × 75 mm, plastic culture tubes (Falcon) and incubated in an atmosphere of 5% CO_2 in air for up to 24 h at 37°C. Drugs in Eagle's MEM were added immediately before culture (20 µl volumes). Cell culture was terminated by centrifugation at 600 *g* for 5 min at 4°C, and the supernatants removed and stored at −20°C before assay. Sterile techniques were employed throughout and no infections were noted during the culture period.

Prostaglandin radioimmunoassay

Prostaglandin-like activity in culture supernatants was measured by radioimmunoassay (Jaffe, Smith, Newton & Parker, 1971) using sheep anti-PGE_2/bovine serum albumin and anti-$PGF_{2\alpha}$/bovine serum albumin antisera. Tritium-labelled prostaglandins (20,000 d/min per 0.1 ml) were equilibrated with the appropriate antiserum (0.1 ml) and unknown or standard (15 to 400 pg/0.1 ml) solutions of prostaglandins in 0.1 M phosphate buffer pH 7.2 at 4°C overnight. Bovine gamma globulin (2.5 mg/0.1 ml) was then added and antibody-bound [^{3}H]-prostaglandin precipitated by the addition of saturated ammonium sulphate solution (0.4 ml). The precipitate was washed once with 50% saturated ammonium sulphate solution (0.8 ml), transferred, with two 0.6 ml aliquots of distilled water, into vials containing 10 ml scintillation fluid (Fisons Toluene Cocktail P: Fisons Emulsifier mixture No. 1, at a ratio of 1:0.5 v/v), and counted in a Packard Tri-Carb liquid scintillation counter. Anti-PGE antiserum cross reacted 100% with PGE_2; 55% with PGE_1; 11% with 13,14-dihydro-PGE_2; 1.5% with $PGF_{2\alpha}$; 1.2% with 15-keto PGE_2; 0.8% with 13,14-dihydro-15-keto-PGE_2; 0.6% with PGA_2; 0.3% with thromboxane B_2; and 0.2% with PGB_2. Anti-PGF antiserum cross-reacted 100% with $PGF_{2\alpha}$; 27% with $PGF_{1\alpha}$; 0.21% with 15-keto-$PGF_{2\alpha}$; 0.16% with 13,14-dihydro-15-keto-$PGF_{2\alpha}$; 0.13% with PGE_2; 0.013% with PGA_2; 0.017% with thromboxane B_2; and 0.016% with PGB_2. Since these antisera are not mono-specific, immunoreactive prostaglandin-like activity was expressed in terms of PGE_2 or $PGF_{2\alpha}$ weight equivalents.

Drugs

The following drugs were used: indomethacin (Merck, Sharp & Dohme); ketoprofen, sodium salt (SPECIA, France); sodium flurbiprofen (Boots); fenoprofen, calcium salt (Eli Lilly); naproxen (Syntex); alclofenac (Berk); azapropazone (E.H. Robins); oxyphenbutazone (Geigy); feprazone, sodium salt (Di Angeli, Italy); phenylbutazone, sodium salt (Di Angeli, Italy); meclofenamic acid (Parke Davis); niflumic acid (Squibb); mefenamic acid (Parke Davis); flufenamic acid (Parke Davis); acetylsalicylic acid (BDH); sodium salicylate (BDH); D-penicillamine (Dista); colchicine (Sigma); dexamethasone sodium phosphate (Decadron; Merck, Sharp & Dohme); prednisolone sodium phosphate (Codelsol; Merck, Sharp & Dohme); prednisolone acetate (Precortisyl; Roussel); methylprednisolone acetate (Depo-Medrone; Upjohn); triamcinolone acetonide (Adcortyl; Squibb); hydrocortisone sodium phosphate (Efcortesol; Glaxo); hydrocortisone sodium succinate (Organon); cortisone acetate (Cortisyl; Roussel) and sodium aurothiomalate (Myochrysin; May & Baker).

[5,6,8,11,12,14,15(n)-^{3}H]-prostaglandin E_2, 160 Ci/mmol, and [9-^{3}H]-prostaglandin $F_{2\alpha}$, 15 Ci/mmol, were purchased from the Radiochemical Centre, Amersham.

Results

Prostaglandin production by macrophage populations

Peritoneal exudate cells obtained from guinea-pigs, 2 to 3 days following intraperitoneal starch injection, consisted of 60 to 80% macrophages, 10 to 30% PMN leucocytes and up to 10% lymphocytes. These macrophage-rich cell populations produced substantial PGE_2-like activity (mean 12.6 ± 2.1 (s.e. mean) ng PGE_2 equivalent/10^6 cells) during 24 h culture. The presence of PGE_2 tentatively identified by bioassay and thin layer chromatography (Gordon, Bray &

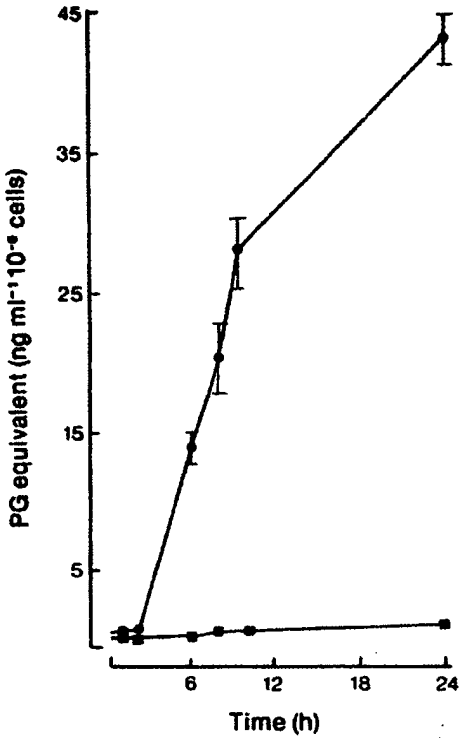

Figure 1 Time course of prostaglandin production by cultured guinea-pig peritoneal exudate macrophages. Macrophages were incubated for the times indicated, and prostaglandin (PG) activities in the culture supernatants determined by radioimmunoassay as described. Each point represents the mean of 6 replicate samples; vertical lines show s.e. means. (●) PGE_2 equivalent; (■) $PGF_{2\alpha}$ equivalent.

Morley, 1976) has been confirmed by combined gas chromatography/mass spectrometry (Nugteren & Van Dorp, Unilever—personal communication). The time course of prostaglandin production by macrophage-rich cell populations is shown in Figure 1. Prostaglandin-like activity was undetectable in supernatant fluids collected up to 2 h; PGE synthesis then proceeded at a fairly constant rate up to 24 h. PGF synthesis was much lower than PGE synthesis. Post-culture viability of cells was >90% viable as estimated by vital dye exclusion. A similar time course and ratio of PGE:PGF production has been reported for cultured human rheumatoid synovial fragments (Kantrowitz, Levine & Robinson, 1975a; Kantrowitz, Robinson, McGuire & Levine, 1975b).

Inhibition of prostaglandin production by indomethacin and other nonsteroidal anti-inflammatory agents

Indomethacin (0.1 ng to 0.1 μg/ml) produced dose-related inhibition of prostaglandin biosynthesis (Figure 2), with 70% inhibition at a concentration of 3.7 ng/ml (range 1.9 to 4.8 ng/ml in 8 experiments). Indomethacin was arbitrarily assigned a potency of 100 and used as a reference compound for comparison with other drugs (Table 1).

Fifteen other nonsteroidal anti-inflammatory agents were examined representing four chemical classes: salicylates (acetylsalicylic acid and sodium salicylate); pyrazolidinediones (phenylbutazone, azapropazone, oxyphenbutazone and feprazone); arylalkanoic acids (ketoprofen, flurbiprofen, fenoprofen, and naproxen) and fenamates (meclofenamic acid, niflumic acid, mefenamic acid and flufenamic acid).

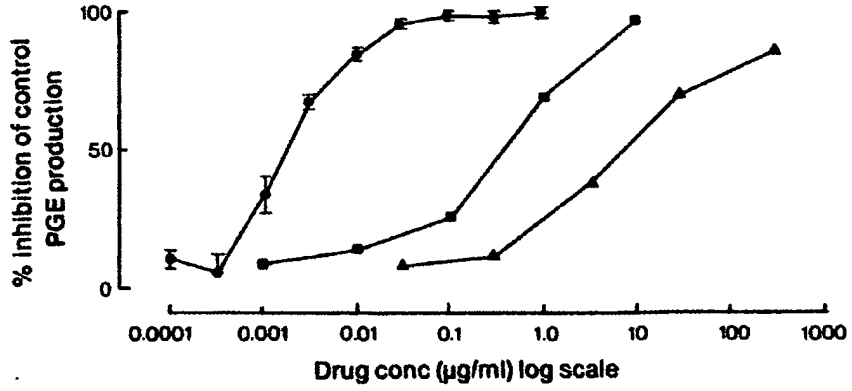

Figure 2 Effect of indomethacin and salicylates on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of indomethacin (●), acetylsalicylic acid (■) and sodium salicylate (▲) at the concentrations shown. For indomethacin each point represents the mean of 12–24 replicate samples from 4 to 8 separate experiments; vertical lines show s.e. mean. For the salicylates each point represents the mean of 6 replicate samples from 2 separate experiments.

Each compound produced dose-related inhibition of macrophage prostaglandin production (Figure 2; Table 1).

Inhibition of prostaglandin production by steroidal anti-inflammatory agents

Dexamethasone (0.1 to 100 ng/ml), prednisolone (1.0 ng to 1.0 μg/ml) and hydrocortisone (10 ng to 1.0 μg/ml), tested as their soluble phosphate esters, exhibited dose-related inhibition of macrophage prostaglandin production. Dexamethasone and prednisolone were, respectively, approximately 8 and 2 times as potent as hydrocortisone, judged on the basis of ID_{70} concentrations of base (Table 1).

In other experiments, dose-related inhibition of prostaglandin production has been observed with triamcinolone acetonide, prednisolone acetate, methylprednisolone acetate and hydrocortisone sodium succinate (Figure 3; Table 1). In contrast, cortisone acetate had no significant effect on prostaglandin production at concentrations up to 1 μg/ml, and stimulated PGE production at concentrations above 10 μg/ml.

Inhibition of prostaglandin production by dexamethasone was unaffected by concurrent addition of insulin (0.1 to 100 mu/ml) suggesting that the action of the anti-inflammatory steroid was not simply a result of depressed glucose utilisation by the cells.

Effects of other anti-inflammatory agents on prostaglandin production

Sodium aurothiomalate (1 μg to 1 mg/ml) produced dose-related inhibition of prostaglandin production, with 70% inhibition being observed at a concentration of 42 μg/ml (Figure 4). In contrast to non-steroidal anti-inflammatory drugs, at concentrations above 10 μg/ml there was some loss of cell viability as measured by vital dye exclusion.

D-Penicillamine, at concentrations up to 100 μg/ml, had no significant effect on macrophage prostaglandin production; however, at 1 mg/ml 20% inhibition was observed (Figure 4). Colchicine increased prostaglandin production at some concentrations with maximal effect at 0.1 μg/ml (no drug control = 6.2 ± 0.7: colchicine 0.1 μg/ml = 11.1 ± 0.4 ng PGE_2 equivalent per 10^6 cells in 24 h) though less

Table 1 Inhibition by anti-inflammatory drugs of macrophage prostaglandin E (PGE) production

Compound	*ID_{70} (ng/ml)*	*Relative potency*
Group 1		
Indomethacin	3.7	100*
Acetylsalicylic acid	1120	0.34
Sodium salicylate	51000	0.006
Azapropazone	530	0.56
Oxyphenbutazone	860	0.35
Feprazone	1400	0.20
Phenylbutazone	3050	0.09
Meclofenamic acid	2.65	183
Niflumic acid	8.6	59.9
Mefenamic acid	20.0	22.7
Flufenamic acid	48.5	9.3
Ketoprofen	28.0	7.0
Flurbiprofen	88.0	2.2
Fenoprofen	405	0.47
Naproxen	750	0.25
Alclofenac	3800	0.05
Group 2		
Dexamethasone (phosphate)	44.0	8.1
Prednisolone (phosphate)	158	2.3
Hydrocortisone (phosphate)	358	1.0†
Triamcinolone (acetonide)	48.1	12.9
Methyl prednisolone (acetate)	57.5	10.7
Prednisolone (acetate)	80.6	7.7
Hydrocortisone (succinate)	1534	0.4

Group 1: relative potency obtained by comparison of the ID_{70} with the ID_{70} for indomethacin* (expressed as 100) determined in the same experiment.
Group 2: relative potency obtained by comparison of the ID_{70} of base with the ID_{70} of hydrocortisone phosphate† (as base) expressed as 1.0 determined in the same experiment.

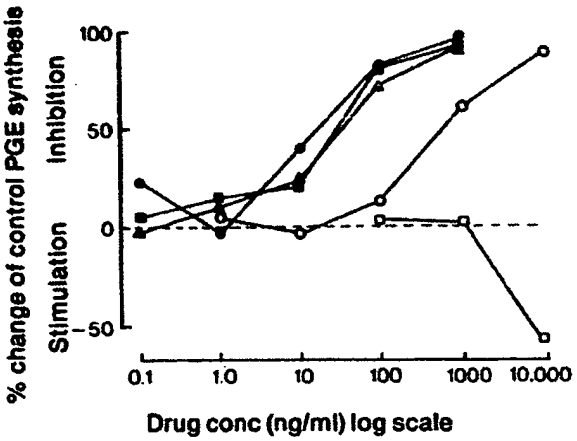

Figure 3 Effect of glucocorticosteroids on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of triamcinolone acetonide (●), methylprednisolone acetate (■), prednisolone acetate (▲), hydrocortisone succinate (○) and cortisone acetate (□) at the concentrations indicated. Each point represents the mean of 6 replicate samples from 2 separate experiments.

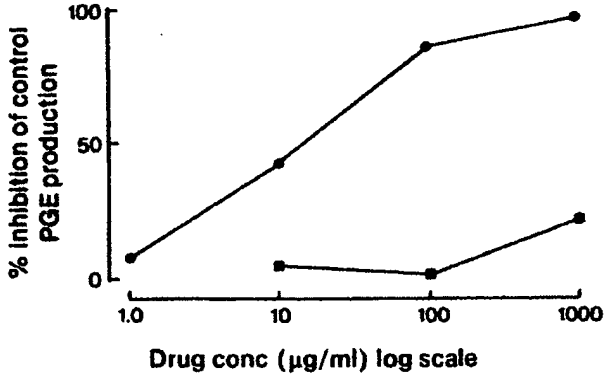

Figure 4 Effect of anti-rheumatic drugs on prostaglandin E (PGE) production by cultured macrophages. Macrophage cultures were incubated for 24 h in the presence of triamcinolone acetonide (●), and D-penicillamine (■) at the concentrations indicated. Each point represents the mean of 6 replicate samples from 2 separate experiments.

stimulation was seen at higher concentrations (colchicine 1.0 μg/ml = 8.3 ± 0.3 ng PGE_2 equivalent per 10^6 cells in 24 h).

Discussion

The observation that macrophages derived from inflammatory exudates produce substantial amounts of prostaglandin E_2-like activity during *in vitro* culture, indicates that this cell type should be considered as a major source of the elevated prostaglandin levels found in inflammation, particularly during chronic stages but also contributing to some earlier manifestations of the inflammatory response. For example, in carrageenin-induced inflammation, although the onset of prostaglandin production apparently coincides with the migration of PMN leucocytes and mononuclear cells into the inflamed site (Anderson *et al.*, 1971), elevated prostaglandin levels persist for longer than PMN leucocyte infiltration and remain elevated during the period of mononuclear cell predominance (Willis, 1969; DiRosa & Willoughby, 1971; DiRosa, Giroud & Willoughby, 1971). In contrast, in urate crystal-induced synovitis, the onset of prostaglandin production and several symptoms of inflammation precede PMN leucocyte accumulation (Glatt *et al.*, 1974); moreover ingestion of urate crystals by synovial-lining macrophages also precedes the onset of vasodilatation, oedema and the subsequent PMN leucocyte accumulation (Schumacher, Phelps & Agudelo, 1974).

Inhibition of prostaglandin synthesis by nonsteroidal anti-inflammatory drugs has been demonstrated in many preparations (Flower, 1974). Dose-related inhibition of guinea-pig peritoneal macrophage prostaglandin synthesis has been demonstrated by 16 acidic non-steroidal anti-inflammatory drugs (Figure 2, Table 1) with at least 70% inhibition at concentrations known to be achieved during anti-inflammatory therapy in man. Hamberg (1972) showed that therapeutic doses of indomethacin, aspirin and sodium salicylate inhibited whole body prostaglandin production in man to a similar degree (greater than 70%).

The sensitivity of macrophage prostaglandin biosynthesis to inhibition by indomethacin is higher than that of homogenates or sub-cellular fractions derived from several tissues (see Flower, 1974) but is comparable to that of intact cells in culture, including human rheumatoid synovium (Kantrowitz *et al.*, 1975a).

An approximate correlation between inhibition of dog spleen prostaglandin synthetase and inhibition of carrageenin-induced rat paw oedema for several nonsteroidal anti-inflammatory agents has been reported, and ID_{50} concentrations were generally less than peak drug levels in man (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972). Our results on macrophages are consistent with this viewpoint, as based upon a comparison of the ID_{50} values obtained from dose-response data. For example, ketoprofen was approximately 17 times more active than naproxen which is in accordance with reports that effective blood levels of these two drugs are in the ranges 2

to 7 μg/ml and 35 to 70 μg/ml respectively (Runkel, Forchielli, Serelius, Chaplin & Segre, 1974; Mitchell, Scott, Kennedy, Brooks, Templeton & Jeffries, 1975). Similarly feprazone was approximately 3.7 times more active than phenylbutazone on macrophage PGE production *in vitro* whilst effective blood levels are respectively 20 to 60 μg/ml and 80 to 140 μg/ml (Burns, Rose, Chenkin, Goldman, Schulert & Brodie, 1953; Chériè-Lingnière, Colombo, Carraba, Ferrari & Gallazi, 1974). Aspirin was approximately 30 times more active than sodium salicylate whilst effective blood levels are 10 to 14 μg/ml and 200 to 300 μg/ml respectively (Rosenthal, Bayles & Fremont-Smith, 1964; Sholkoff, Eyring, Rowland & Riegelman, 1967).

Although there is an observed correlation between inhibition of *in vitro* macrophage prostaglandin production and clinically effective blood levels within each chemical class of non-steroidal anti-inflammatory agent tested, no account has been taken of factors such as plasma protein binding, which is important *in vivo* because only free drug can reach the sites of action and exert a pharmacological effect (Koch-Weser & Sellers, 1976). However, pharmacokinetic studies with indomethacin have shown that symptomatic relief (analgesia) of inflammatory joint disease in man is more closely associated with drug levels in synovial fluid than in blood (Emori, Champion, Bluestone & Paulus, 1973; Brooks, Bell, Lee, Rooney & Dick, 1974). Nonetheless the level of indomethacin attained in synovial fluid (approximately 0.7 μg/ml) following the oral administration of therapeutic dosage in man (Emori *et al.*, 1973) is sufficient to inhibit by more than 90%, prostaglandin production by guinea-pig macrophages (Figure 2) even allowing for a high degree (95%) of protein binding. Similarly, synovial free drug concentrations achieved following clinically active doses of three other drugs which have been studied, ketoprofen (Mitchell *et al.*, 1975), feprazone (Chériè-Lignière *et al.*, 1974) and aspirin (Rosenthal *et al.*, 1964; Sholkoff *et al.*, 1967) are sufficient to inhibit macrophage prostaglandin synthesis to a comparable degree. The ratio of acetylsalicylate to salicylate in synovial fluid after analgesic doses is approximately 1:20–30, and the potency ratio inhibiting macrophage prostaglandin production (30:1) is not inconsistent with this.

Corticosteroids have been reported to produce no inhibition of prostaglandin biosynthesis (Vane, 1971; Smith & Willis, 1971; Ferreira, Moncada & Vane, 1971), small degrees of inhibition at high concentrations (Greaves & McDonald-Gibson, 1972a & b) or marked inhibition at therapeutic concentrations (Lewis & Piper, 1975; Gryglewski, 1975; Kantrowitz *et al.*, 1975b; Tashjian, Voelkel, McDonough & Levine, 1975) depending on the prostaglandin synthesizing preparation used. Prostaglandin production by cultured macrophages has been shown in this study to be inhibited by seven anti-inflammatory steroid preparations, with complete inhibition again being achieved at concentrations attained during anti-inflammatory therapy in man. The potency ratios of dexamethasone, prednisolone and hydrocortisone (Table 1) are in reasonable agreement with their clinical anti-inflammatory activities (Goodman & Gilman, 1975). The sensitivity of macrophage prostaglandin production to inhibition by these three drugs closely resembles that of human rheumatoid synovium *in vitro* (Kantrowitz *et al.*, 1975b).

It is also noteworthy that inhibition by hydrocortisone of macrophage prostaglandin production was evident at concentrations within the physiological range (Goodman & Gilman, 1975). The lack of inhibition of macrophage prostaglandin production by cortisone acetate suggests that these cell populations are unable to convert cortisone to its biologically active derivative hydrocortisone.

Of the two drugs tested with anti-rheumatic activity, D-penicillamine was essentially inactive at concentrations achieved during anti-rheumatic treatment (100 μg/ml plasma with a daily dose of 1 g of drug) (Figure 4). Thus inhibition of prostaglandin biosynthesis seems unlikely to be important in the mechanism of action of this drug. Aurothiomalate caused a dose-related inhibition of PGE production at concentrations known to be attained in serum and synovial tissue of patients on maintenance gold therapy (Lorber, Atkins, Chang, Lee, Starrs & Bovy, 1973; Rubenstein & Dietz, 1973; Grahame, Billings, Laurence, Marks & Wood, 1974). However, the observed dose-related increase in cell death at concentrations above 20 μg/ml seen in some experiments may contribute to the observed inhibition of prostaglandin synthesis, since prostaglandin synthesis was a feature of viable macrophages. Colchicine, which has a selective anti-inflammatory effect in acute attacks of gouty arthritis, but which is not therapeutically useful in other types of arthritis, enhanced macrophage prostaglandin biosynthesis with maximal effect at a concentration of 0.1 μg/ml. Colchicine (0.1 μg/ml) has also been reported to increase the production of prostaglandins by human rheumatoid synovium (Robinson, Smith & Levine, 1973). This effect of colchicine occurs at concentrations greater than those achieved during normal therapy (Wallace, Omojuku & Ertel, 1970). Prostaglandin production during urate crystal-induced synovitis was also enhanced by colchicine, although PMN leucocyte infiltration and the oedema of inflammation were simultaneously depressed (Glatt *et al.*, 1974).

In these experiments the effects of established anti-inflammatory drugs have been examined on the spontaneous *in vitro* prostaglandin production by macrophages which had been activated *in vivo*. We have previously demonstrated that such cell populations

increase prostaglandin production in response to lymphocyte activation products (Gordon *et al.*, 1976). More recently, other workers have shown that macrophage prostaglandin production may also be enhanced by agents that activate the complement system via the alternative pathway (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977; Glatt, Wagner & Brune, 1977; Wahl, Olsen, Sandberg & Mergenhagen, 1977).

The striking similarities in prostaglandin production by cultured guinea-pig macrophages and human rheumatoid synovia, and their qualitative and quantitative responses to pharmacological agents, support the recent suggestion that the macrophage is the cell type responsible for prostaglandin production by synovial fragments (Dayer, Krane, Russell & Robinson, 1976; Bray & Gordon, 1976). Therefore, it seems likely that guinea-pig macrophages may provide a relevant, simple and inexpensive *in vitro* system for the evaluation of analgesic-anti-inflammatory drugs on the prostaglandin component of the inflammatory response. Furthermore, the potential exists for simultaneous investigation of drug effects on two other important inflammatory mediators released by these mixed cell populations viz. lymphocyte activation products (lymphokines) and macrophage lysosomal enzymes.

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Immunology

Class II major histocompatibility complex molecules of murine dendritic cells: Synthesis, sialylation of invariant chain, and antigen processing capacity are down-regulated upon culture

(epidermal Langerhans cells/dendritic cell differentiation/T-cell sensitization)

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ABSTRACT **Dendritic cells (DCs), such as Langerhans cells (LCs) of the epidermis and the DCs of lymphoid organs such as spleen, are potent antigen presenting cells. DCs express high levels of major histocompatibility complex (MHC) class II molecules, but, partly because of the low numbers of primary DCs in any tissue, there has been no detailed study of the biochemistry of their class II molecules. This information may be needed to help explain recent findings that DCs process native protein antigens when freshly isolated from epidermis and spleen. Processing ceases during culture, yet a strong accessory function for activating resting T cells develops. We studied immunoprecipitates of DC class II and invariant chain (I_i) molecules by two-dimensional gel electrophoresis. We found that (*i*) freshly isolated LCs synthesize large amounts of class II and I_i polypeptides; (*ii*) I_i molecules that are known to be involved in antigen processing display an unusually large number of sialic acids in fresh LCs; (*iii*) with culture, class II and I_i synthesis decreases dramatically and has virtually ceased at 3 days; and (*iv*) the turnover of class II in pulse/chase experiments is slow, being undetectable over a 12- to 32-hr culture period, whereas the turnover of I_i is rapid. We conclude that MHC class II molecules of DCs do not seem to be qualitatively unique. However, the regulation of class II and I_i expression is distinctive in that biosynthesis proceeds vigorously for a short period of time and the newly synthesized class II remains stably on the cell surface, whereas I_i turns over rapidly. This may enable DCs to process and retain antigens in the peripheral tissues such as skin and migrate to the lymphoid organs to activate T cells there.**

Dendritic cells (DCs) represent a system of abundantly major histocompatibility complex (MHC) class II-expressing leukocytes. They occur in nonlymphoid organs, blood, afferent lymph, and lymphoid tissues (1). Two states of differentiation can be distinguished (2, 3). "Immature" DCs, exemplified by freshly isolated epidermal Langerhans cells (LCs), are weak stimulators of resting T cells both in the allogeneic mixed leukocyte reaction (4, 5) and in polyclonal responses such as oxidative mitogenesis, concanavalin A mitogenesis, and anti-CD3 mitogenesis (6). These fresh LCs (7, 8) as well as fresh DCs from spleen (9), however, are efficient in processing native protein antigens for MHC class II-restricted presentation to presensitized peptide-specific T cells (*in vivo*-primed T cells, clones, T–T hybridomas). Upon 1–3 days of culture in macrophage- or keratinocyte-conditioned medium, or in culture medium supplemented with granulocyte/macrophage colony-stimulating factor, their functional properties become inverted. "Mature" DCs lose the capacity to process exogenous antigens but at the same time acquire the ability to sensitize resting T cells (2, 3). The loss of processing function is paralleled by the loss of acidic organelles such as endosomes (10), which are known as the compartments where class II encounters processed antigen (11–13).

Since MHC class II molecules are the essential elements of antigen processing and presentation, we studied in detail the composition and the chemical nature of class II polypeptides and associated invariant chains (I_i) in the course of DC maturation/differentiation in culture.

MATERIALS AND METHODS

Mice. BALB/c mice were purchased from Charles River Wiga, Sulzfeld, F.R.G., and were used between 6 and 10 weeks of age.

Cells. Fresh [day 0 LCs (LCd0)] and cultured [day 1 and day 3 LCs (LCd1 and LCd3)] LCs were prepared and enriched from ear skin as described (4, 14, 15). DCs were obtained from spleen (16). Thioglycollate-elicited peritoneal macrophages were induced to express MHC class II by recombinant murine interferon γ (Stratech, London) (17). In addition, a macrophage line (P388D1) and a pre-B-cell line (18-81) were used.

Immunolabeling Procedures. Epidermal sheets were prepared as described (18). Single cell suspensions were attached to multiwell microscopic slides coated with poly-L-lysine (50 μg/ml; type VII; Sigma). Cells were briefly fixed with 4% paraformaldehyde. Antibody and washing solutions contained 0.02% saponin (Sigma) to enhance penetration. Monoclonal antibody (mAb) In1 (19), rat IgG2b anti-murine invariant (I_i) chain, reacting with intracytoplasmic I_i31 and I_i41 chains (20) was used. Binding of In1 was visualized with biotinylated anti-rat immunoglobulin (not cross-reactive with mouse immunoglobulin; Vector, Burlingame, CA) followed by streptavidin fluorescein isothiocyanate (Amersham). To identify LCs, this incubation sequence was extended by rat immunoglobulin to block free anti-rat binding sites, and tetramethylrhodamine B isothiocyanate-conjugated anti-I-$A^{b,d}$ (B21-2; TIB229 from the American Type Culture Collection).

Metabolic Labeling. Cells were washed three times in methionine-free RPMI 1640 medium (GIBCO). Cells (1×10^6) were resuspended in 60 μl of methionine-free RPMI 1640 medium and 100 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (SJ204; Amersham) was added. With partially enriched

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Abbreviations: EC, epidermal cell; DC, dendritic cell; I_i, invariant chain; LC, epidermal Langerhans cell; LCd0, freshly isolated LC; LCd1, 1-day cultured LC; LCd3, 3-day cultured LC; MHC, major histocompatibility complex; mAb, monoclonal antibody.

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LCd0, up to 40 × 10^6 total epidermal cells (ECs) were incubated in proportional volumes and radioactivities. After 90–120 min at 37°C, the tubes were filled up with 15 ml of warm culture medium and incubated for another hour. Then cells were washed and lysed. For pulse/chase experiments, 30 × 10^6 ECs (containing 6 × 10^6 LCd0) and 30 × 10^6 macrophages were labeled with 40 μCi per 1 × 10^6 ECs in 200 μl for 15 min at 37°C. After three washes in complete medium, ECs were cultured as described above for 12 and 32 hr. Macrophages (15 × 10^6) were cultured for the same periods in Teflon beakers.

Cell-Surface Iodination. Cell-surface proteins were labeled by the lactoperoxidase method (21).

Immunoprecipitation and Two-Dimensional Gel Electrophoresis. This was done as described in detail previously (22), using mAb In1 (see above), rat mAb 17/227, anti-I-A^d/E^d, and rat mAb NLDC-145, anti-DC-specific antigen (23). On two-dimensional gels, proteins were separated by charge using nonequilibrium pH gradient gel electrophoresis in the first (horizontal) dimension and by size using reducing NaDodSO$_4$/PAGE in the second (vertical) dimension. To achieve a semiquantitative comparison between the various cell populations, equal numbers of class II-expressing cells were labeled and immunoprecipitated and gels were scanned by densitometry (Laser Densitometer, Molecular Dynamics, Sunnyvale, CA).

Functional Assays. Oxidative mitogenesis assays (4, 14) and antigen-specific hybridoma assays (8, 10) were done as described.

RESULTS

Functional Properties of Immature and Mature DCs. Freshly isolated LCs are weak stimulators of resting T cells (4) but excellent processors of native protein as determined with peptide-specific T-cell clones (7) or hybridomas (refs. 8 and 10; Table 1). After 2–3 days of culture, LCs selectively lose processing capacity; in the same time, however, they become much more efficient in stimulating resting T cells (Table 1).

Expression of MHC Class II Molecules and Associated I_i on DCs. It has been shown that immature DCs—i.e., resident LCs—express substantial levels of MHC class II antigens (4, 16, 24). We inspected epidermal sheets by immunohistochemistry for the presence of class II and associated I_i. Both molecules are coexpressed in LCs but are not detectable in surrounding keratinocytes (Fig. 1 *A* and *B*).

Upon culture of LCs, the surface expression of MHC class II molecules is rapidly (i.e., within hours) increased up to 10-fold (4, 24, 25) and resembles class II expression on splenic and thymic DCs. In contrast, the expression of intracellular I_i chain decreased upon 3-day culture of LCs as visualized with mAb In1 (Fig. 1 *C–H*; ref. 8). Equally low amounts of cytoplasmic I_i were immunostained in spleen DCs (data not shown).

High Rate of I_i and Class II Biosynthesis in Immature DCs and Its Decrease upon Their Maturation in Culture. Although class II and I_i genes are located on different chromosomes and their structural genes are not related under immune-activated conditions, their expression is tightly co-regulated (26). Two-dimensional separation of metabolically labeled class II and I_i immunoprecipitates reveals that freshly isolated LCs synthesize large amounts of class II and I_i polypeptides (Fig. 2 *A* and *F*). When LCs were cultured for different times and their biosynthesis was analyzed, profound changes were observed. The rate of synthesis of both class II and I_i molecules dropped sharply (Fig. 2 *B–D*; ref. 8). After 1 day of culture, this became evident and within 3 days LCs had virtually stopped class II and I_i production. Spleen DCs synthesize considerably less class II and I_i than fresh LCs, albeit not as little as LCd3 (Fig. 2*E*). The down-regulation of class II/I_i production in cultured LCs was selective, because the same cells actively synthesized another protein—namely, the DC antigen immunoprecipitated by mAb NLDC-145 (8, 23) (data not shown).

Unusual Expression and Maturation of I_i in Immature LCs. The I_i gene encodes two polypeptides—I_i31 and I_i41 (27). In B cells, I_i41 usually represents 10% or less of I_i (19, 28) (Fig. 3*E*). In freshly isolated LCs, however, the proportion of I_i41 in relation to I_i31 is unusually high (Fig. 3*A*). Densitometric analysis of the gels depicted in Fig. 3 gave I_i31/I_i41 ratios of 1.3 (43% I_i41) for fresh LCs, 3.4 (23% I_i41) for spleen DCs, 2.3 (30% I_i41) for macrophages, and 10.4 (9% I_i41) for B cells.

Upon intracellular transport of class II and I_i polypeptides to trans-Golgi compartments, their glycan side chains are sialylated (29). I_i chains from freshly isolated LCs acquire an unusual number of sialic acids (Fig. 3*A*; up to 17 acidic spots), which increase the molecular weight of I_i31 up to 45,000. Similarly, I_i41 is intensely sialylated. As a consequence of sialylation, some I_i chains are more acidic than class II α chains (Fig. 3*A*, far right). Upon maturation of LCs, the

Table 1. Reciprocal expression of antigen processing and T-cell sensitizing capacities in fresh and cultured LCs

Antigen presenting cells	Dose of antigen presenting cells						
	10^4	3 × 10^3	10^3	3 × 10^2	10^2	3 × 10^1	10
Hybridoma assay	Proliferation of CTLL-2						
A. fLCs −	6.2	5.4	5.9	5.1	5.0	5.4	5.2
B. fLCs + myo	129.3	172.9	199.3	145.6	121.1	52.1	17.8
C. cLCs −	1.0	1.5	2.5	1.3	2.8	2.1	2.1
D. cLCs + myo	2.7	1.0	2.0	2.4	2.3	1.9	1.6
Oxidative mitogenesis	Proliferation of T cells						
E. 0-hr LCs	—	44.9	15.5	5.9	2.7	—	—
F. 12-hr LCs	—	45.9	19.3	6.5	2.2	—	—
G. 32-hr LCs	—	180.4	144.0	52.8	15.6	—	—
H. 12-hr MAs	—	8.6	4.2	2.1	1.6	—	—
I. 32-hr MAs	—	8.7	4.9	3.0	2.4	—	—

The hybridoma assay was as follows. Antigen processing capacity was determined by measuring interleukin 2 production of myoglobin-peptide-specific hybridoma 11.3.7 by means of CTLL-2 indicator cells (rows A–D). Note that freshly isolated LCs (fLCs) efficiently activate the hybridoma in the presence of sperm whale myoglobin protein (myo) (row B). Cultured LCs (cLCs) have lost this capacity (row D). Oxidative mitogenesis was as follows. LCs and peritoneal macrophages (MAs) cultured for 0, 12, and 32 hr were used to stimulate periodate-modified resting T cells. Note that 12-hr LCs (row F), which express levels of class II equivalent to those of 32-hr LCs (row G) (5, 24), are as weak stimulators as are freshly isolated LCs (row E). Class II-positive macrophages do not activate resting T cells (rows H and I). This experiment was done in parallel to the labeling experiment in Figs. 4 and 5. Results are expressed as cpm × 10^{-3}.

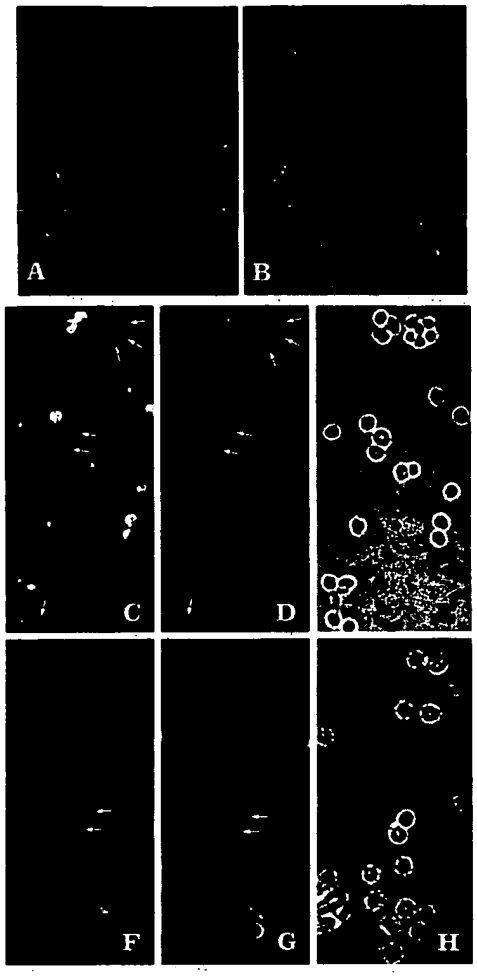

FIG. 1. Expression of I_i molecules by murine epidermal LCs. Epidermal sheets (*A* and *B*) were double-stained with mAb In1, anti-I_i (*A*), and mAb B21-2, anti-I-$A^{b,d}$ (*B*). Note that all class II-expressing cells also bear I_i and vice versa. Enriched populations of fresh (*C–E*) and 3-day-cultured LCs (*F–H*) attached to poly-L-lysine-coated slides were double-stained with mAbs In1 (*C* and *F*) and B21-2 (*D* and *G*). The expression of I_i chains decreases with culture and is almost absent in LCs cultured for 3 days (*C* vs. *F*), whereas class II expression increases (*D* vs. *G*). Note that all photographs were exposed and developed identically to allow for a semiquantitative comparison of fluorescence intensities. Arrows, keratinocytes. (*E* and *H*) Corresponding phase-contrast pictures. (×100.)

number of sialic acid residues bound per I_i molecule decreases. Cultured LCs as well as spleen DCs, a macrophage, and a B-cell line carry 9–11 acidic spots per I_i31 chain as opposed to up to 17 in freshly isolated LCs (Fig. 3 *B–E*). The molecular weight of the most sialylated I_i31 chains of cultured LCs remains below 40,000 and their negative charge resembles that of class II α chains. Class II molecules were always less sialylated (three or four acidic spots) than I_i chains (Fig. 3). It is emphasized that they, when opposed to actin as an internal reference standard, did not strongly vary in their charge (i.e., sialylation) between LCd0, LCd1, spleen DCs, macrophages, and B cells.

Comparison of Molecular and Functional Properties of Immature and Mature DCs. The exclusive functional property of mature DCs to sensitize resting T cells was frequently attributed to the nature of class II molecules on these cells (30, 31). However, biochemical analysis of surface radioiodinated LCs (data not shown) revealed no unusual pattern of surface class II molecules of mature DCs as compared to macrophages or B cells. Alternatively, fresh LCs were metabolically pulse labeled and chased for 12 or 32 hr to ensure

FIG. 2. Biosynthesis of class II and invariant chain polypeptides in LCs and DCs. LCd0 (*A*, *B*, and *F*), LCd1 (*C*), LCd3 (*D*), and spleen DCs (*E*) were metabolically labeled for 1 hr with [^{35}S]methionine and lysates were immunoprecipitated with anti-class II (*A–E*) and anti-I_i (*F*) mAbs. To demonstrate the decreasing intensities of the two-dimensional pattern, the gels in *B–E* were exposed to x-ray films for 25 days. In addition, the two-dimensional gels in *A* and *F* were exposed for 1 day. Class II chains are denoted with Aβ, Eβ, Eα, Aα (*A*) or with Ia (*C–F*). I_i41 and I_i31 are coprecipitated invariant chains. Note the marked decrease in biosynthesis upon culture of LCs (*B–D*). Assignment of the spots was done by using defined class II/I_i transfectants (22). A, actin.

expression of radiolabeled molecules on the cell surface (32). The same populations were also used to study antigen-presenting cell function. Anti-class II immunoprecipitates of 12-hr chased LCs (i.e., functionally immature LCs; Table 1) or 32-hr chased LCs (i.e., functionally mature LCs; Table 1) gave virtually identical two-dimensional gels (Fig. 4 *A* and *B*). There was also no striking qualitative difference to class II of peritoneal macrophages (Fig. 4).

Turnover of MHC Class II and I_i Molecules. This was studied in pulse/chase experiments of metabolically labeled cells. Class II molecules of LCs were more stable than those of macrophages: after a 12- or 32-hr chase period, the amounts of precipitable class II molecules were similar in LCs indicating that they had not decreased during this

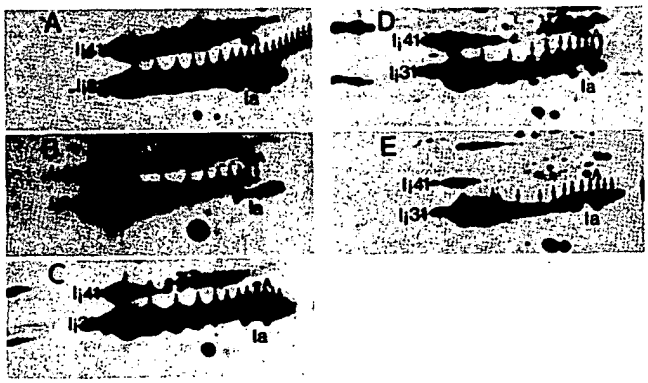

FIG. 3. Glycosylation patterns of I_i. Two-dimensional gels of I_i immunoprecipitates were exposed to x-ray films for various times to obtain similar intensities of invariant chains. (*A*) LCd0, 2 days. (*B*) LCd1, 25 days. (*C*) Spleen DC, 25 days. (*D*) Macrophage line P388D1 [preincubated with interferon γ (50 units/ml) to induce class II molecules], 20 days. (*E*) Pre-B-cell clone 18-81H6 (class II-positive variant of the 18-81 cell line), 10 days. The number of acidic spots of the I_i31 chain is marked by arrows. The ratio of I_i41/I_i31 is highest in fresh LCs (*A*) and lowest in B cells (*E*). Bands visible on the far left of *C* and *D* are ^{14}C molecular weight standards of M_r 27,000 and 46,000. Ia, class II molecules; A, actin.

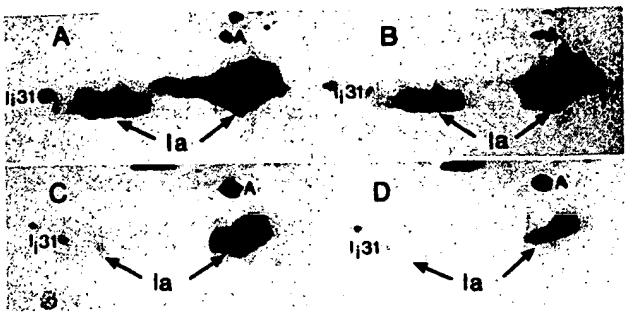

FIG. 4. Pulse/chase labeling of class II molecules in LCs and peritoneal macrophages. Freshly isolated LCs (*A* and *B*) and peritoneal macrophages (*C* and *D*) were pulse labeled for 15 min with [^{35}S]methionine and subsequently cultured for 12 hr (*A* and *C*) or 32 hr (*B* and *D*). Class II polypeptides (Ia and arrows) were immunoprecipitated with mAb 17/227. Note that only low amounts of I_i are present because of its rapid turnover and dissociation from class II. A, actin.

interval (Fig. 4 *A* and *B*). In contrast, markedly less class II was precipitated from macrophages after a 32-hr chase than after a 12-hr chase, suggesting a shorter half-life (Fig. 4 *C* and *D*). Densitometric scans of the gels in Fig. 4 demonstrated a 55% reduction of precipitable material (i.e., class II molecules) in macrophages over a period of 20 hr but no reduction in LCs. I_i molecules showed a different turnover. In freshly prepared LCs, they are synthesized in extremely large amounts as compared to other cell types (Fig. 2 *B–E*). The amount of I_i decreased sharply during the chase period, which is consistent with a short half-life of I_i (compare Fig. 5 *A* and *B* with Fig. 2*F*). It is remarkable that in LCs—as opposed to macrophages—I_i41 was relatively more stable than I_i31. Although initially more I_i31 than I_i41 was made by fresh LCs (Fig. 2*F*), most of the I_i31 had disappeared after a pulse/chase of 12 or 32 hr, whereas I_i41 remained precipitable with In1 (Fig. 5 *A* and *B*). In macrophages, neither chain was precipitable after the chase (Fig. 5 *C* and *D*).

DISCUSSION

DCs can efficiently activate resting T lymphocytes and are therefore key antigen presenting cells (1). It is unclear whether and how this capacity relates to the nature of MHC class II molecules. Also, the phenomenon of DC maturation/differentiation in culture (loss of antigen processing capacity and acquisition of stimulatory function) (3) needed to be studied in detail at the level of class II biochemistry. This

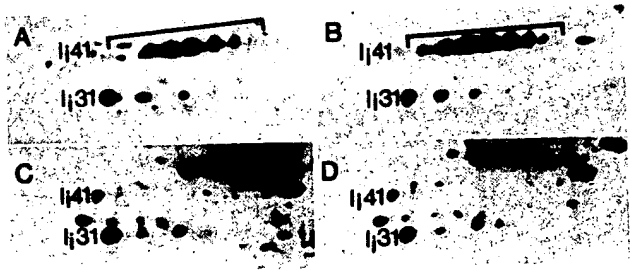

FIG. 5. Pulse/chase labeling of invariant chains in LCs and peritoneal macrophages. Cells were prepared and labeled as in Fig. 4. I_i chains were immunoprecipitated with In1. In both cultured LCs (*A* and *B*) and in macrophages (*C* and *D*) only marginal amounts of sialylated I_i31 chains and small amounts of unsialylated precursors are present. This indicates a high turnover rate of I_i31. In contrast, sialylated I_i41 in LCs is more abundant than I_i31, although synthesis of I_i31 in fresh LCs exceeds that of I_i41. Associated class II is hardly detectable. A spot to the left of I_i41 and I_i31 of macrophages is distinct from I_i chains and was not identified.

report confirms and extends recent work by Puré *et al.* (8) by using two-dimensional analyses and by focusing on structural features of the invariant chain.

Biochemistry of Class II and I_i Molecules of Immature DCs—Significance for Antigen Processing. LCs *in situ* or freshly isolated from the epidermis have some capacity to endocytose (8, 33, 34). They are equipped with acidic organelles like endosomes (10), the necessary cellular compartments for antigen processing (11, 12). The high rate of class II synthesis gives immature DCs the possibility to charge a large number of class II molecules with immunogenic peptides. The concomitant abundant synthesis of I_i, which is also involved in antigen processing (22, 26), would ensure that the peptide binding groove (35, 36) of all *de novo*-synthesized class II molecules is protected until the class II/I_i complex reaches the organelle where it encounters the antigen, presumably the acidic endosome (11, 12). The unusually high degree of I_i sialylation in fresh LCs gives these polypeptides a strong negative charge. This could promote trafficking/targeting and protease stability of the class II/I_i complexes, thereby facilitating their entry into the acidic processing organelles (27, 37). The significance of the high I_i41/I_i31 ratio is unknown at present. Taken together, these biochemical features may explain the extraordinary antigen-processing capacity of immature DCs as compared to other types of antigen presenting cells (7–9, 33).

Biochemistry of Class II and I_i Molecules of Mature DCs—Significance for T-Cell Sensitization. The cellular mechanism responsible for the unique capacity of mature DCs to sensitize resting T cells is presumably their ability to bind T cells in an antigen-*in*dependent fashion (38). It has not been possible so far to block this type of DC–T-cell interaction with antibodies to defined adhesion molecules (39–41). Moreover, the high density of class II molecules on mature DCs does not account for this binding property (5). Therefore, one may either postulate a hitherto unknown "clustering molecule" or, alternatively, obvious differences in class II molecules. Addressing the latter possibility, analysis of two-dimensional gels did not reveal such differences between class II molecules of antigen presenting cells that can (cultured LCs, spleen DCs) or cannot (fresh LCs, macrophages, B cells) bind T cells antigen independently. Low sialylation of surface class I and II has been discussed to be responsible for the immunostimulatory power of mature DCs (30, 31). However, we found no differences of class II sialylation patterns of immature and mature DCs and of B cells. Also, neuraminidase treatment of fresh LCs (F.K., N.R., and G.S., unpublished data) and macrophages (42) did not endow these cells with a T-cell sensitizing capacity. Thus, the putative molecule responsible for the initial antigen-independent binding of mature DCs to resting T cells remains unknown. Our data make it seem unlikely that it is merely a modification of class II.

Regulation of Class II and I_i Synthesis. Using flow cytometry it was shown that the increase in class II expression upon culture of LCs was not dependent on the cytokines granulocyte/macrophage colony-stimulating factor and tumor necrosis factor α (14). Also, interleukin 4 and/or interferon γ do not appear to be responsible cytokines because the up-regulation of class II expression occurs also in the absence of these factors (14). Cytokine effects on the synthesis of class II/I_i as measured by metabolic labeling of DCs have not yet been investigated.

LC Culture as a Physiologic Model for Antigen Presentation *in Vivo*. There is evidence that antigen-laden LCs can migrate from the skin to the draining lymph node (43, 44). It is tempting to speculate that before or during this migration LCs shut off class II/I_i synthesis, thereby preventing the possibility that by continuous processing the cells displace those immunogenic peptide/class II complexes that they have

formed while still in the epidermis. Indeed, both types of mature DC studied, cultured LCs and spleen DCs, have virtually stopped making class II and I_i. Their I_i molecules (Figs. 1 and 5) as well as their acidic organelles (10) rapidly decreased. In contrast, class II molecules were relatively stable in LCs as compared to murine B-cell lines and macrophages (45, 46). This very slow turnover of class II would make DCs well suited to carry antigenic peptides bound to class II to the lymphoid organs over a period of several days and efficiently present them to the T cells there. Indeed, it was recently shown that antigen-pulsed DCs can retain immunogenic peptide for at least 2 days *in vitro* (8) and *in vivo* (33).

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Immunology

Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection

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ABSTRACT **Recent data implicates a role for the CD40–CD40 ligand (CD40L) pathway in graft rejection. One potential mechanism is direct costimulation of T cells through CD40L. Alternatively, the ability of CD40 stimulation to induce CD80 (B7-1) and CD86 (B7-2) expression on antigen-presenting cells (APCs) has led to the hypothesis that the role of CD40–CD40L interactions in transplant rejection might be indirect, i.e., to promote the costimulatory capacity of APCs. Here, we have used a murine vascularized cardiac allograft model to test this hypothesis. Treatment of the recipients with donor splenocytes and a single dose of anti-CD40L mAb induces long-term graft survival (>100 days) in all animals. This is associated with marked inhibition of intragraft Th1 cytokine [interferon γ and interleukin (IL) 2] and IL-12 expression with reciprocal up-regulation of Th2 cytokines (IL-4 and IL-10). In untreated allograft recipients, CD86 is strongly expressed on endothelial cells and infiltrating mononuclear cells of the graft within 24 hr. In contrast, CD80 expression is not seen until 72 hr after engraftment. Anti-CD40L mAb has no detectable effect on CD86 up-regulation, but almost completely abolishes induction of CD80. However, animals treated with anti-CD80 mAb or with a mutated form of CTLA4Ig (which does not bind to CD86) rejected their cardiac allografts, indicating that blockade of CD80 alone does not mediate the graft-prolonging effects of anti-CD40L mAb. These data support the notion that the role of CD40–CD40L in transplant rejection is not solely to promote CD80 or CD86 expression, but rather that this pathway can directly and independently costimulate T cells. These data also suggest that long-term graft survival can be achieved without blockade of either T cell receptor-mediated signals or CD28–CD86 engagement.**

It is well-accepted that T cells require costimulatory signals for optimal activation (1, 2). At present, CD28 is the best-characterized costimulatory receptor on T cells (3, 4). Its known ligands, CD80 (B7-1) and CD86 (B7-2), are expressed on activated antigen-presenting cells (APCs) (3, 4). Blockade of CD28–B7 interactions has been shown to inhibit a number of immune responses *in vitro* and *in vivo*, including transplant rejection, induction of graft versus host disease, and autoimmune syndromes (for review, see ref. 5).

Recently, other costimulatory pathways have been characterized (1). One which has been the subject of intense study is that of CD40–CD40 ligand (CD40L) (6, 7). CD40, a member of the tumor necrosis factor (TNF)-receptor family is expressed on the surface of B cells. T cell activation induces expression of a molecule of the TNF family known as CD40L, and binding of CD40L to CD40 during cognate T–B interactions provides B cell help (for review, see ref. 8). CD40 is expressed on other APCs (macrophages and dendritic cells) as well (9). Recent studies showing that CD40 engagement induces the expression of CD80 and CD86 (10, 11) suggest an indirect function of this pathway, i.e., to promote CD28-mediated costimulation. However, it also has been reported that engagement of CD40L provides a direct costimulatory signal to the T cell (12). Here, we have addressed this question by examining the ability of anti-CD40L mAb to prevent murine cardiac allograft rejection and modulate the expression of B7 molecules *in vivo*.

MATERIALS AND METHODS

Murine Cardiac Allografts. C57BL/6 (H-2^b) and BALB/c (H-2^d) mice aged 6–8 weeks were purchased from The Jackson Laboratory and housed in pathogen-free conditions. Cardiac allografts from BALB/c donors into C57BL/6 recipients were placed in an intraabdominal location (13). Graft function was assessed daily by palpation. Animals received mAbs or fusion proteins (at a dose of 200 μg unless otherwise stated) by intravenous injection at the time of transplantation or 2 days after engraftment. In some instances, animals also received an intravenous injection of 5×10^6 donor splenocytes at the time of transplantation. Rejection was defined as the day of cessation of palpable heartbeat, and was verified by autopsy and selective pathological examination. Loss of graft function within 48 hr of transplant was considered a technical failure (<10% on average), and these animals were omitted from further analysis.

Monoclonal Antibodies and Fusion Proteins. The anti-CD40L mAb MR-1 and a control hamster Ig were gifts of Randy Noelle (Dartmouth Medical School, Hanover, NH). The anti-CD80 mAb 16-10A1 was a gift of Gary Gray (Repligen, Boston). The anti-CD86 mAb GL1 was obtained from American Type Culture Collection. The fusion protein CTLA4Ig has been described (14). The fusion protein CTLA4IgY100F was produced by introducing a phenylalanine residue at position 100 in place of tyrosine using PCR primer-directed mutagenesis. In brief, *in vitro* and *in vivo* studies indicate that CTLA4IgY100F binds CD80 with similar avidity as does CTLA4Ig, but has at least a 200-fold lower avidity for CD86. *In vitro*, CTLA4Ig has detectable binding (by fluorescence-activated cell sorter) to CD86-transfected Chinese hamster ovary cells at concentrations as low as 10-30 ng/ml, whereas CTLA4IgY100F fails to bind to CD86-transfected Chinese hamster ovary cells at concentrations as high as 100 μg/ml. Both CTLA4Ig and CTLA4IgY100F bind equivalently to CD80-transfected Chinese hamster ovary cells (R.P. and

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Abbreviations: APC, antigen-presenting cell; CD40L, CD40 ligand; DST, donor-specific transfusion; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon.

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P.S.L., unpublished work). The half-life of CTLA4IgY100F in mice is similar to that of CTLA4Ig. CTLA4IgY100F effectively blocks CD80-dependent responses (32).

Immunopathology. Portions of each graft were snap-frozen and stored at −70°C until sectioning, or formalin-fixed and paraffin-embedded for standard light microscopy. Isotype-matched control mAbs and rat and hamster mAbs against mouse proteins were purchased from PharMingen, unless specified. These consisted of mAbs for CD80 (16-10A1) and CD86 (GL1); cell surface markers expressed by all leukocytes (CD45, 30F11.1), T cells (CD5, 53-7.3), B cells (CD45R/B220, RA3-6B2), monocytes (CD11b, M1/70), natural killer cells (NK1.1, PK136), and granulocytes (Gr-1, RB6-8C5); the activation antigens VCAM-1 (CD106, 429) and interleukin (IL)-2R (CD25, 3C7); and the cytokines IL-2 (S4B6), IL-4 (11B11), interferon (IFN) γ (R4-6A2), IL-10 (JES5-2A5), and TNF-α (MP6-XT22), plus a polyclonal antibody to IL-12 (R & D Systems). Cryostat sections were fixed either in paraformaldehyde-lysine-periodate for demonstration of cell surface antigens or in acetone for localization of cytokines, and were stained by a four-layer PAP method as described (15, 16). Each graft was analyzed at three or more levels, with counts of $CD45^+$ leukocytes in 10-20 fields per section (expressed as mean ± SD of cells per high power field). The specificity of labeling was assessed using isotype-matched mAbs or purified Ig. In addition, the specificity of cytokine staining was confirmed by overnight mAb absorption with recombinant cytokines (IL-2, IL-4, IL-10, and IFN-γ, obtained from PharMingen) prior to immunohistologic labelling of selected cytokine-rich day 3 allografts (15, 16).

RESULTS

Effect of anti-CD40L mAb on Cardiac Allograft Survival. We first studied the effect of a blocking hamster anti-murine CD40L mAb on cardiac allograft rejection. As shown in Fig. 1, administration of a single dose of anti-CD40L mAb on the day of transplantation significantly delayed rejection in all recipients, and led to indefinite graft survival in the majority (five of seven recipients). Previously, using a rat model of cardiac allografts, we found that the use of donor-specific transfusion (DST) consisting of donor-type splenocytes, was synergistic with CTLA4Ig in preventing graft rejection (17).

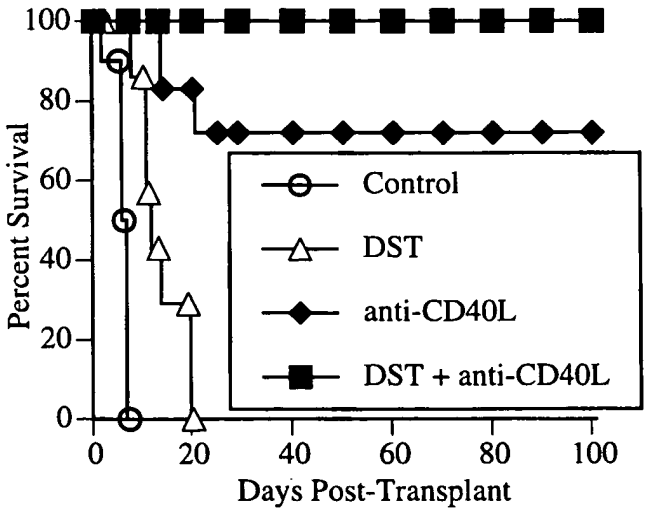

FIG. 1. Effect of anti-CD40L mAb on cardiac allograft survival. BALB/c hearts were transplanted into C57BL/6 recipients. Allograft recipients were treated with either a control Ig or anti-CD40L mAb (200 μg by intravenous injection) at the time of transplantation. Selected recipients also received a transfusion of 5×10^6 donor lymphocytes (DST) at the time of transplantation.

Similar results were reported recently with murine islet allografts, in which a 7-week course of treatment with anti-CD40L mAb (1 week pre- and 6 weeks posttransplant) alone prevented rejection in 40% of animals, whereas the addition of a single DST led to indefinite graft survival in 96% of the recipients (17). To determine if DST similarly improved graft survival in animals treated with anti-CD40L mAb, mice received a transfusion of 5×10^6 donor-type splenocytes at the time of transplantation (Fig. 1). DST alone had a slight graft-prolonging effect, although most grafts were rejected within 2 weeks, and all were lost by 3 weeks. The combination of DST and anti-CD40L mAb was clearly synergistic, with all animals maintaining allograft function beyond 150 days.

Effect of CD40L mAb on Intragraft Cytokine Expression. We next examined the effect of anti-CD40L mAb on the pattern of intragraft cytokine gene expression (Fig. 2 and Table 1). At day 3 after transplantation, control grafts (treated with DST plus control Ig) contained mononuclear cells expressing IL-2 and IFN-γ, but essentially no cells producing IL-4 or IL-10 were detectable. In contrast, while there was an equivalent mononuclear infiltrate in the grafts of DST plus anti-CD40L mAb-treated animals (Table 1), these cells lacked detectable IL-2 or IFN-γ, but showed a striking induction of both IL-4 and IL-10 expression. In additional experiments, we found that the intensity and pattern of expression of IL-2, IL-4, IL-10, and IFN-γ, as well as the extent of the mononuclear cell infiltrate, were identical in animals treated with a control Ig alone compared with animals treated with DST plus control Ig (data not shown). Furthermore, animals treated with anti-CD40L mAb alone (without DST) had identical findings as those treated with anti-CD40L mAb plus DST (data not shown). Thus, the effects of DST plus anti-CD40L mAb on cytokine expression are a specific result of blockade of CD40L. They are neither the result of DST alone nor require the concomitant use of DST. Animals treated with anti-CD40L mAb also had reduced intragraft expression of the proinflammatory cytokines IL-12 and TNF-α (Table 1). Interestingly, anti-CD40L mAb had no effect on the expression of vascular cell adhesion molecule-1, a ligand for Very Late Antigen-4 (Table 1).

Expression of CD80 and CD86 During the *in Vivo* Response to Alloantigen. Given the role of the CD40L-CD40 pathway in modulating the expression of CD80 and CD86 *in vitro* (10, 11), and the known importance of B7 molecules in graft rejection (3, 5), it was important to characterize the normal pattern of CD80 and CD86 expression during an alloimmune response *in vivo*, and to determine the effects of anti-CD40L mAb on these parameters. For these experiments, cardiac allografts were examined by immunohistologic labeling at serial time points posttransplant (Fig. 3). Neither CD80 nor CD86 molecules were detected in significant amounts in the cardiac tissue before transplantation, with only rare and scattered interstitial dendritic cells and an occasional endothelial cell being labeled (Fig. 1, day 0). By 24 hr after transplantation of cardiac allografts, CD86 was densely expressed on virtually all endothelial cells and interstitial dendritic cells. CD86 expression was not increased in control cardiac isografts (Fig. 3) within the limits of detection, suggesting that up-regulation of this molecule is occurring due to specific immune recognition of foreign major histocompatibility complex proteins and the ensuing immune response, and not merely as a result of a nonspecific inflammation secondary to the "trauma" of transplantation (tissue manipulation, ischemia, etc.). In contrast to the prompt up-regulation of CD86 expression, CD80 expression remained unchanged (i.e., weak dendritic cell labeling) within the graft both 24 and 48 hr after transplantation. A striking difference was seen 3 days after transplantation however, at which point CD80 (as well as CD86) was densely expressed on most dendritic cells and infiltrating macrophages, i.e., a population of cells that expressed CD86 at 24 hr. CD80 expression on endothelial cells was more focal and never

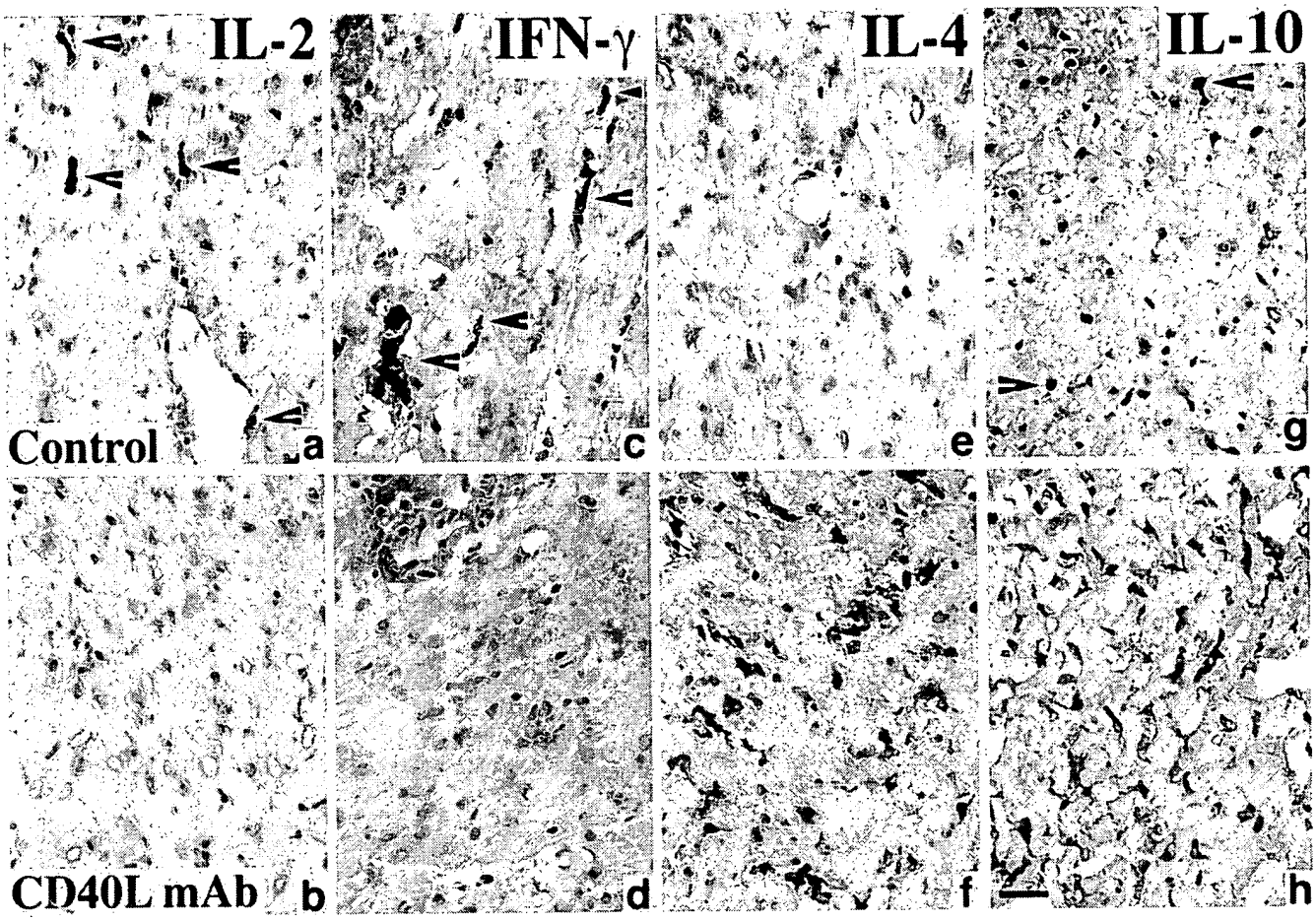

FIG. 2. Effect of anti-CD40L mAb on intragraft cytokine gene expression. C57BL/6 recipients of BALB/c cardiac allografts were treated with either DST plus 200 μg of control Ig (*Upper*) or DST plus 200 μg of anti-CD40L mAb (*Lower*) at the time of transplantation. The animals were killed after 3 days and the hearts were stained for expression of IL-2, IFN-γ, IL-4, and IL-10 as described. Arrowheads (*a* and *c*) indicate the typical mononuclear cell labeling for IL-2 and IFN-γ confined to control grafts, whereas grafts from anti-CD40L mAb-treated recipients showed dense mononuclear and some adjacent endothelial cell labeling for IL-4 (*f*) and IL-10 (*h*). Cryostat sections, hematoxylin counterstain. (Bar = 50 microns.)

appeared to be as strong as CD86 expression on those cells, which remained high even at 3 days.

Effect of CD40L mAb on the Expression of B7 Molecules. To determine whether anti-CD40L mAb affected CD80 or CD86 expression, the animals were killed at day 3 (a time when control animals express both CD80 and CD86 in their grafts; see Fig. 3) for immunopathologic assessment (Fig. 3). By comparison with untreated animals, it can be seen that DST plus control Ig by itself had no observable effect on the pattern or intensity of CD80 or CD86 expression. Interestingly, however, there was a differential effect of anti-CD40L mAb treatment on the expression of CD80 and CD86. While anti-CD40L mAb treatment had no detectable effect on the expression of CD86, it almost completely abrogated the induction of CD80 expression, with treated animals having only a small number of residual $CD80^+$ cells in their grafts, similar to what was seen before transplantation or in control isografts. This was not due to alterations in the populations of cells available for examination, as both control-Ig and anti-CD40L-treated animals had equivalent mononuclear infiltrates as assessed by cell morphology and immunohistochemical staining for T cells, macrophages, natural killer cells, and B cells

Table 1. Immunopathology of day 3 cardiac allografts

Feature	Control Ig plus DST	Anti-CD40L mAb plus DST
Leukocyte infiltration*	Moderate, multifocal infiltrate of T cells (<25%) and monocytes (>75%)	Same
$IL\text{-}2R^+$ cells	10–20% of MNCs	<1% of MNCs
IL-2, IFN-γ	5–10% of MNCs	Negative
IL-4, IL-10	<1% of MNCs	>50% of MNCs and ECs
IL-12, TNF-α	20–50% of MHC and focal ECs	<1% of MNCs
VCAM-1	Most ECs	Same

Comparable data were seen in mice given control Ig alone and mice given DST plus control Ig. Data reflect evaluation of 10-20 fields per graft and three grafts per group. Normal hearts lacked any of these features apart from the presence of small numbers of $CD45^+$ resident dendritic cells and a rare $VCAM\text{-}1^+$ endothelial cell. MNC, mononuclear cell; EC, endothelial cell; VCAM-1, vascular cell adhesion molecule-1.

*Control Ig plus DST grafts contained 43 ± 11 $CD45^+$ leukocytes per high power field, and anti-CD40L mAb plus DST grafts contained 39 ± 12 leukocytes per high power field (P = not significant).

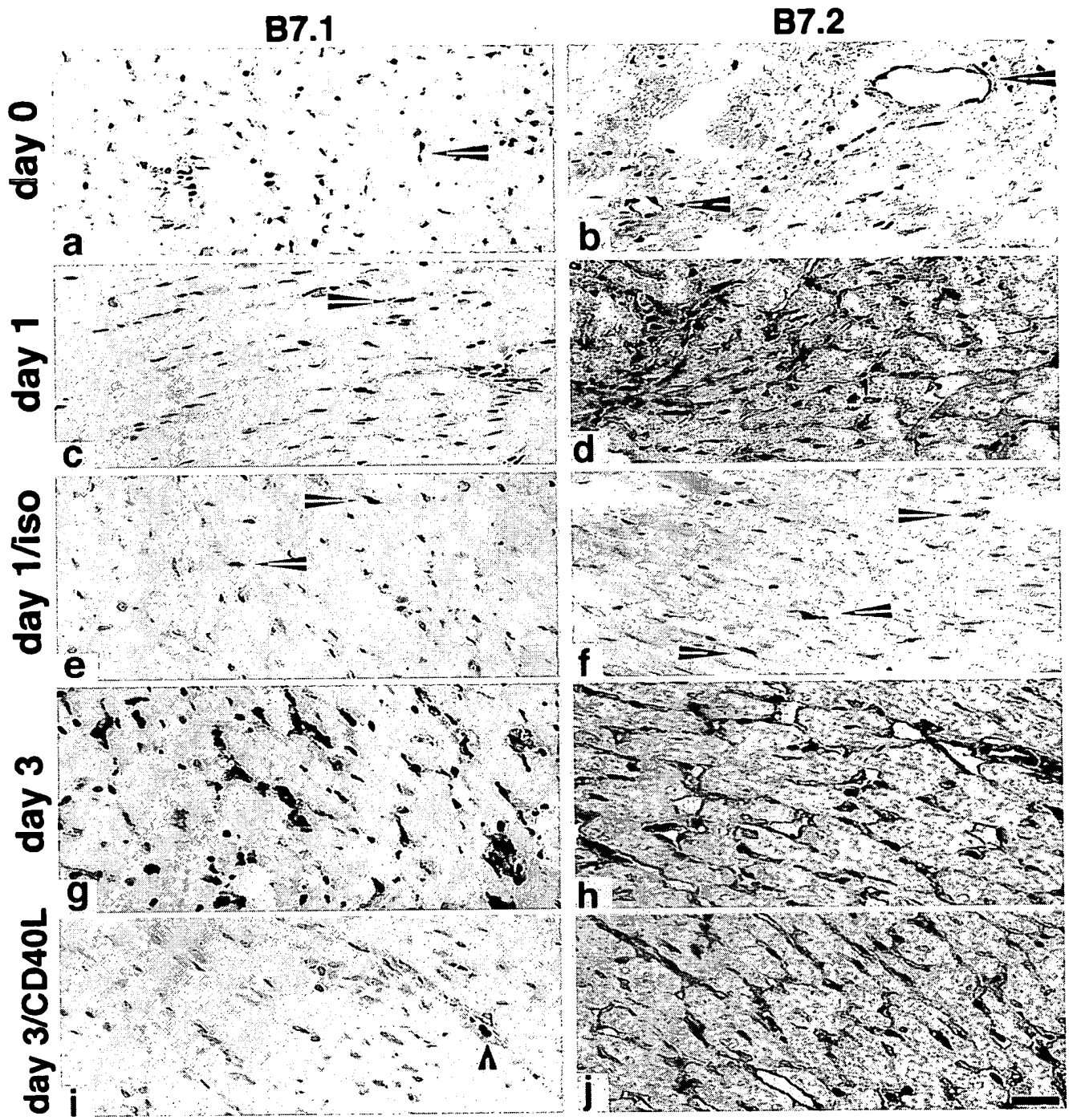

FIG. 3. Serial changes in B7-1 and B7-2 expression within murine cardiac allografts. BALB/c hearts transplanted into C57BL/6 recipients were harvested after 1 or 3 days and sections stained for the expression of CD80 (B7-1) or CD86 (B7-2). Day 0 panels represent a harvested donor heart immediately before transplantation. An isograft control (BALB/c donor into BALB/c recipient) was harvested on day 1. Arrowheads in *a* and *c* indicate the weak labeling for B7-1 that is restricted to interstitial dendritic cells in the first day posttransplantation, whereas by day 3, dense mononuclear and some capillary endothelial cell labeling is seen (*g*). In contrast, B7-2 is expressed by occasional graft endothelial cells (arrowheads in *b*) but is rapidly and densely up-regulated in allografts within 24 hr (*d*) but not in a day 1 isograft (*f*). Where indicated, the recipients were treated with DST plus 200 μg of anti-CD40L mAb at the time of transplantation (*i* and *j*). DST plus CD40L mAb administration blocked up-regulation of B7-1, without affecting graft cellularity (compare *g* and *i*). The dense endothelial expression of B7-2 was not modulated by DST plus anti-CD40L mAb (compare *h* and *j*). DST plus control Ig did not affect the marked increase in intragraft B7-1 seen at day 3 in control allografts (data not shown). Cryostat sections, hematoxylin counterstain. (Bar = 50 microns.)

(Table 1, and data not shown). The lack of effect of anti-CD40L mAb on CD86 expression also serves as a control for cells that are potentially capable of expressing CD80. Therefore, as assessed by immunohistochemical staining of the grafts, we find that the ability of anti-CD40L treatment to prevent acute cardiac allograft rejection, and to promote long-term survival, is associated with selective blockade of CD80 induction. Furthermore, the staining patterns for CD80 and CD86 were not altered by DST alone, and were identical in animals treated with anti-CD40L mAb alone, as in animals receiving anti-CD40L mAb plus DST (data not shown). Thus, as with the findings regarding intragraft cytokine staining (Fig. 2), the effects of anti-CD40L mAb on intragraft staining of CD80 and CD86 are not dependent upon the use of DST.

Table 2. Effects of CD80 and CD86 blockade on graft survival

Treatment	Cardiac allograft survival
Control Ig	6, 7, 7, 7, 7, 7, 8, 8, 8, 8, 8
DST	11, 12, 12, 13, 19, 20, 20
CTLA4Ig + DST	>150 (n = 12)
Anti-B7-1 + DST	20, 22, 23, 42, 42
CTLA4IgY100F + DST	5, 5, 7, 11, 16
Anti-CD86 + DST	7, 25, 28, 100+, 100+
CTLA4IgY100F + anti-CD86 + DST	>150 (n = 4)

BALB/c hearts were transplanted into C57BL/6 recipients. Allograft recipients were treated with a control Ig, anti-CD80 mAb, or anti-CD86 mAb (200 μg by intravenous injection), CTLA4Ig (200 μg), or CTLA4IgY100F (600 μg) 2 days after transplantation. Selected recipients also received a transfusion of 5×10^6 donor lymphocytes (DST) at the time of transplantation.

Effect of Selective CD80- and CD86-Blockade on Allograft Survival. The data in Fig. 3 suggested the hypothesis that the mechanism by which anti-CD40L mAb prevented graft rejection was through inhibiting the induction of CD80 on APCs. Therefore, we next tested the ability of selective blockade of CD80 or CD86 to prevent rejection. As seen in Table 2, and consistent with our previous studies in rats (5, 17), a single dose of CTLA4Ig (given on day 2) plus DST induced indefinite graft survival in all cardiac allograft recipients. In contrast, DST plus anti-CD80 mAb minimally delayed rejection and was unable to induce long-term survival. Blockade of CD86 alone with mAb induced long-term survival in a minority of recipients.

Although the anti-CD80 mAb used in the studies above (16-10A1) clearly blocks CD28 binding (18), it has been suggested that intact anti-CD80 mAbs might have uncharacterized positive signaling effects on CD80-expressing cells (19). Therefore, in additional experiments, we used a mutated form of CTLA4Ig, called CTLA4IgY100F, in which the tyrosine at position 100 was replaced by a phenylalanine. This mutation causes CTLA4IgY100F to retain binding activity for CD80 but abolishes binding to CD86. An advantage of this reagent over Fab fragments of anti-CD80 mAb are its relatively longer half-life (53 hr) compared with expected half-life of Fab fragments *in vivo*. CTLA4IgY100F had no effect, either at the same dose as CTLA4Ig (200 μg, data not shown) or at a 3-fold higher dose (600 μg, Table 2). The inability of CTLA4IgY100F to prevent rejection was not due to failure to adequately block CD80. Table 2 shows that CTLA4IgY100F and anti-CD86 mAb synergize in preventing allograft rejection, indicating that CTLA4IgY100F is an effective competitive inhibitor for CD80 binding. Thus, selective blockade of CD80 *in vivo* is unable to replicate the immunosuppressive effects of anti-CD40L mAb.

DISCUSSION

To our knowledge, this is the first report describing the pattern of expression of the costimulatory ligands CD80 and CD86 *in vivo* during the response to a vascularized organ allograft. We find that both molecules are up-regulated as part of a specific immune response (rather than as a result of the transplant procedure itself), and that CD86 is expressed significantly earlier than CD80. The expression pattern of the two molecules is distinct, in that CD86 appears to be quite a bit more prominent on endothelial cells than does CD80. The endothelium is the first site of contact of host T cells with recipient tissue in the case of vascularized grafts. This, coupled with the known ability of endothelium to express major histocompatibility complex class II molecules, makes it likely that endothelial cells play a very prominent role in the early stages of alloactivation *in vivo*.

Many previous studies attest to the importance of CD80 and CD86 in transplant rejection (5, 17, 20–22). More recent evidence suggests a role for the CD40-CD40L pathway in these responses as well (6–8, 23). This might be as a direct T cell costimulator (12, 24) or via induction of CD80 and CD86 on B cells (10, 11). In addition, CD40 is expressed on macrophages, and ligation of CD40 has been shown to potentiate macrophage production of nitric oxide and selective monokines such as IL-12, and to enhance macrophage cytotoxicity (25).

Our data indicate that blockade of CD40L by administration of a single dose of mAb at the time of transplantation is able to induce long-term survival of vascularized cardiac allografts in ≈70% of murine recipients. The addition of DST leads to indefinite graft survival in all animals, although the mechanisms by which DST augment the effects of anti-CD40L mAb are not known. Parker *et al.* (23) have shown that anti-CD40L antibody combined with DST blocked rejection of murine islet allografts, although in that system prolonged administration (2–7 weeks) of the antibody was required. Our model, one of vascularized organ transplantation, introduces an additional level of complexity.

Recently, Larsen *et al.* (26) have shown that anti-CD40L mAb alone could prevent murine cardiac allograft rejection in the majority of recipients. In their studies, CTLA4Ig, given at the time of transplantation, did not prevent ultimate graft rejection, consistent with our own previous reports regarding the need to delay administration of CTLA4Ig (17). However, combination of anti-CD40L mAb and CTLA4Ig initiated at the time of transplantation was synergistic, leading to long-term survival in all animals (27). While anti-CD40L mAb alone did not effect the expression of T cell cytokines or of B7 molecules, the combination of CTLA4Ig plus anti-CD40L inhibited the expression of IL-2, IL-4, IL-10, and IFN-γ, as determined by reverse transcription–PCR. Transcripts for CD80 and CD86 were only minimally affected. This is in contrast to our own data, where blockade of CD40L alone induced a Th2 immune deviation in association with a loss of CD80 expression. The reasons for this discrepancy are not immediately apparent, but may relate to their use of multiple doses of anti-CD40L mAb to prevent rejection, or may be due to the use of different detection methods [protein detection by immunohistochemistry in the present study versus mRNA detection by RT-PCR in Larsen *et al.* (26)].

It is important to note that our results of costimulatory molecule and cytokine expression were all obtained using immunohistochemistry. An advantage of this technique is the ability to detect protein itself, and the preservation of tissue architecture, allowing for spatial localization of the relevant gene products. While we cannot exclude low residual CD86 expression or a small affect on CD80 expression in the grafts of anti-CD40L-treated animals, the large alterations in CD80, CD86, and cytokine gene expression seen in the present study are real, and seem likely to be physiologically meaningful.

Endothelial cells, when activated, are immunogenic, expressing major histocompatibility complex class I and II molecules, adhesion receptors, and costimulatory molecules. Recently, three groups have shown that CD40 is expressed on endothelial cells and that ligation of CD40 on endothelial cells *in vitro* up-regulates intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 (28–30). In that system, neither CD80 nor CD86 was induced by CD40 ligation of endothelium (28). While that signal may not by itself induce these molecules, our data clearly indicate that, in the case of alloimmune responses, CD40-mediated signals are required for CD80 induction *in vivo*.

In is also interesting to note that treatment with anti-CD40L mAb was able to prevent rejection in the face of abundant CD86 expression throughout the graft endothelium and graft infiltrating APCs. This result implies that long-term graft survival can be achieved without blockade of either T-cell antigen receptor-mediated signals or the CD28 pathway. Thus,

while CD28-mediated costimulation may be necessary for graft rejection, our data suggest that it is not sufficient (at least not when CD86 is the ligand), and that signals mediated through CD40L-CD40 interactions are required as well. Although extremely unlikely, it should be noted that we cannot exclude a direct effect of anti-CD40L mAb on TCR or CD28 signaling.

In our studies, prolongation of graft survival by anti-CD40L mAb was accompanied by immune deviation toward Th2 cytokines, and by specific down-regulation of CD80 expression without an effect on CD86 expression. Recently, Stüber *et al.* (31) reported that anti-CD40L Ab prevented Th1-mediated inflammatory colitis by blocking the secretion of IL-12, an effect observed in our study as well (Table 1). Whether or not the loss of Th1 and induction of Th2 cytokines we observed is responsible for the graft prolonging effects of anti-CD40L mAb is not known. This pattern of selective cytokine sparing is associated with enhanced graft survival in a variety of models; however, a causal role for Th2 cells in transplantation tolerance has yet to be established.

Our studies define the effects of CD40L-blockade on the expression of the T cell costimulators known to be required for transplant rejection (i.e., CD80 and CD86), and suggest that the effects of anti-CD40L mAb on these molecules alone cannot account for its tolerogenic effects in organ transplantation. We cannot, however, completely exclude the possibility that anti-CD80 mAb and CTLA4IgY100F provided only incomplete blockade of CD80, relative to the inhibition seen with anti-CD40L mAb. Further experiments using CD80-knockout mice will be required to answers this question as well as to address the potentially distinct roles of CD80 on donor compared with recipient cells, and on T cells versus APCs.

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A mechanism for the antiinflammatory effects of corticosteroids: The glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial–leukocyte adhesion molecule 1 and intercellular adhesion molecule 1

(neutrophils/endothelium/inflammation)

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ABSTRACT **Corticosteroids are the preeminent antiinflammatory agents although the molecular mechanisms that impart their efficacy have not been defined. The endothelium plays a critical role in inflammation by directing circulating leukocytes into extravascular tissues by expressing adhesive molecules for leukocytes [e.g., endothelial–leukocyte adhesion molecule 1 (ELAM-1) and intercellular adhesion molecule 1 (ICAM-1)]. We therefore determined whether corticosteroids suppress inflammation by inhibiting endothelial expression of adhesion molecules for neutrophils (polymorphonuclear leukocytes). Preincubation of endothelial cells with endotoxin [lipopolysaccharide (LPS), 1 μg/ml] led to a 4-fold increase in subsequent adherence of polymorphonuclear leukocytes ($P < 0.0001$, $n = 10$) to endothelial cells, an increase that was markedly attenuated when endothelial cells were treated with dexamethasone ($IC_{50} < 1$ nM, $P < 0.0001$, $n = 6$ or 7) during preincubation with LPS. Moreover, the steroid receptor agonist cortisol (10 μM), but not its inactive metabolite tetrahydrocortisol (10 μM), diminished LPS-induced endothelial cell adhesiveness. Further evidence that the action of dexamethasone was mediated through ligation of corticosteroid receptors [human glucocorticoid receptors (hGRs)] was provided by experiments utilizing the steroid antagonist RU-486. RU-486 (10 μM), which prevents translocation of ligated hGR to the nucleus by inhibiting dissociation of hGR from heat shock protein 90, completely aborted the effect of dexamethasone on adhesiveness of endothelial cells ($P < 0.0005$, $n = 3$). Treatment of endothelial cells with LPS (1 μg/ml) stimulated transcription of ELAM-1, as shown by Northern blot analysis, and expression of membrane-associated ELAM-1 and ICAM-1, as shown by quantitative immunofluorescence (both $P < 0.001$, $n = 9$). Dexamethasone markedly inhibited LPS-stimulated accumulation of mRNA for ELAM-1 and expression of ELAM-1 and ICAM-1 ($IC_{50} < 10$ nM, both $P < 0.001$, $n = 4$–9); inhibition of expression by dexamethasone was reversed by RU-486 (both $P < 0.005$, $n = 4$–6). As in the adhesion studies, cortisol but not tetrahydrocortisol inhibited expression of ELAM-1 and ICAM-1 (both $P < 0.005$, $n = 3$ or 4). In contrast, sodium salicylate (1 mM) inhibited neither adhesion nor expression of these adhesion molecules. These studies suggest that antagonism by dexamethasone of endotoxin-induced inflammation is a specific instance of the general biological principle that the glucocorticoid receptor is a hormone-dependent regulator of transcription.**

Although glucocorticoids are among the most potent and widely used antiinflammatory agents, the mechanisms by which they reduce inflammation are unknown. Various hypotheses have been proposed; these include "allosteric" effects on enzymes (1), redirection of lymphocyte traffic (2), direct inhibition of various phospholipases (3), induction of such proteins as lipocortin (4), inhibition of the transcription of various cytokines and metalloproteases (5–14), and our own earlier suggestion that glucocorticoids stabilize lysosomal and other cellular membranes (15, 16). However, none of these hypotheses is sufficient to account for the well-known pharmacologic effects of glucocorticoids in humans: leukocytosis (17), inhibition of leukocyte recruitment to inflamed areas (18, 19), retention of lymphocytes in the lymphatic circulation with shrinkage of peripheral lymph nodes, and the promotion of microbial infection (2).

Recent studies have suggested that endothelial cells can direct the traffic of leukocytes into inflamed and infected areas (heterotypic adhesion) via the regulated expression of surface adhesive molecules [e.g., GMP140, endothelial–leukocyte adhesion molecule 1 (ELAM-1), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule (VCAM) (20–23)]. In a complementary fashion, leukocytes also express proteins [CD11a-c/CD18, L-selectin, or lectin/epidermal growth factor cell adhesion molecule 1 (LECAM-1)] on their surface that mediate their specific localization to sites of inflammation (24, 25). Agents that modulate the interaction of leukocytes with the endothelium may, therefore, possess potent antiinflammatory properties.

We now present data compatible with the hypothesis that glucocorticoids—at nanomolar concentrations—inhibit the expression of adhesive molecules ELAM-1 and ICAM-1 by endotoxin-activated endothelial cells and thereby interfere with the traffic of leukocytes into inflamed areas. Pretreatment of endothelial cells with corticosteroids prevents endothelial cells from becoming more adhesive for neutrophils [polymorphonuclear leukocytes (PMNs)] and diminishes stimulated expression of ICAM-1 and ELAM-1, molecules critical for neutrophil adhesion. Moreover, these data make it likely that corticosteroids regulate ELAM-1 at the transcriptional level.

MATERIALS AND METHODS

Materials. Lipopolysaccharide (LPS, *Salmonella typhimurium*) was obtained from Calbiochem and *N*-formylmethionylleucylphenylalanine was obtained from Vega Biochemi-

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Abbreviations: ELAM-1, endothelial–leukocyte adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; PMN, polymorphonuclear leukocyte; LPS, lipopolysaccharide; HUVEC, human umbilical vein endothelial cell; IL, interleukin; TNF, tumor necrosis factor; hGR, human glucocorticoid receptor; FITC, fluorescein isothiocyanate.

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cal. Collagenase, dexamethasone, hydrocortisone, tetrahydrocortisol, and sodium salicylate were obtained from Sigma. RU-486 was a gift to Herbert Samuels (Roussel-Uclaf). Medium 199, RPMI 1640, and fetal bovine serum were obtained from GIBCO. Ficoll/Hypaque was purchased from Nyegaard (Oslo). All other salts and reagents were of the highest quality that could be obtained.

Monoclonal Antibodies. In these studies the monoclonal antibodies used included antibodies directed against ICAM-1 (84H10, AMAC, Westbrook, ME), ELAM-1 (BMA 4D10, Accurate Chemicals, Westbury, NY, and H18/7, a generous gift of Michael Bevilacqua, San Diego), MOPC21, and UPC10, nonbinding isotype controls. Fluorescein-labeled anti-IgG, goat anti-mouse fluorescein isothiocyanate (GAM-FITC) was obtained from Coulter Immunology. Endothelial cells were also stained with rhodamine-labeled *Ulex europaeus* agglutinin I (Vector Laboratories); all monoclonal antibody studies were performed in ice-cold phosphate-buffered saline (PBS) with 0.02% sodium azide and 0.025% bovine serum albumin.

Culture of Endothelial Cells. Human umbilical vein endothelial cells (HUVECs) were cultured as described (26). All experiments were performed on endothelial cells in their third passage.

Incubation of Endothelial Cells with Pharmacologic Agents. Endothelial cells were stimulated by incubation with LPS (1 μg/ml) in a medium consisting of RPMI 1640/10% fetal bovine serum for 4 hr at 37°C in a 5% CO_2 atmosphere with or without other agents as indicated. The monolayers were then washed three times.

PMN Adherence to Endothelial Monolayers (Heterotypic Adherence). PMNs (150,000 per well), isolated from whole blood as described (27), suspended in RPMI 1640 medium, were added to monolayers of endothelial cells and incubated for 10 min at 37°C in a 5% CO_2 atmosphere and adherence was determined as described (28). Pretreatment of unstimulated HUVECs with each of the agents used did not affect basal PMN adherence (data not shown). The highest concentration of diluent used (ethanol, 0.1%) also had no effect on PMN adherence to LPS-stimulated endothelium.

In some experiments PMNs were labeled with ^{111}In and after incubation of labeled PMNs with endothelium for 10 min at 37°C adherence was determined by a previously described method (29) and expressed as % adherence.

Expression of ELAM-1 and ICAM-1. After incubation for 4 hr with various stimuli and agents the HUVECs were removed from wells by exposure to EDTA (0.01%, wt/vol) in PBS for 10 min at 37°C in a 5% CO_2 atmosphere followed by gentle scraping with a rubber policeman and trituration. Cells were resuspended in ice-cold saline containing sodium azide (0.02%) and saturating concentrations of antibodies, incubated for 30 min at 4°C, washed and counterstained with fluorescein-labeled anti-IgG for 30 min at 4°C, washed, and fixed in formaldehyde (3.7% in PBS). HUVECs were then analyzed with a FACScan (Becton Dickinson) (30). In these experiments the fluorescence of cells stained with an isotype control antibody (MOPC21) was 33 ± 13.

Analysis of Message for ELAM-1. HUVECs were incubated without or with stimuli in the presence and absence of dexamethasone (0.1 μM) for 3 hr at 37°C. Following treatment with collagenase/EDTA [0.1%/0.01% (wt/vol) 10 min at 37°C] cells were suspended and washed, and mRNA was isolated by use of the FastTrack kit following the instructions provided (Invitrogen, San Diego). After electrophoresis through agarose the mRNA was transferred to nitrocellulose and hybridized with full-length ^{32}P-labeled cDNA probes for ELAM-1 (generously supplied by Tucker Collins) and actin (Stratagene) under conditions of high stringency. Labeled cDNA for ELAM-1 hybridized with a single band of 3.65 kilobases (kb) and labeled cDNA for actin hybridized with a single band of 2.0 kb. Autoradiograms were prepared and then analyzed by laser densitometry (31).

Statistical Analysis. The significance of differences among and between experimental treatment groups was determined by means of the appropriate level of analysis of variance and determination of separate post-hoc variances by means of the CSS Software (Statsoft, Tulsa, OK) using an IBM-compatible computer.

RESULTS

Glucocorticoids Prevent Adhesion of PMNs to LPS-Stimulated Endothelial Cells (Heterotypic Adhesion). Activation of HUVECs with LPS or cytokines [interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α)] causes endothelial cells to bind unstimulated PMNs more avidly (20, 21, 30, 32). When we treated HUVECs with LPS (1 μg/ml) we found, as expected, that ≈4-fold more PMNs adhered to treated than untreated HUVECs (50 ± 3 vs. 13 ± 2 PMNs per high-power field, $P < 0.0001$). Dexamethasone inhibited PMN adhesion to the LPS-stimulated HUVECs in a dose-dependent manner ($IC_{50} < 1$ nM, $P < 0.005$, Fig. 1).

Glucocorticoid Receptors Mediate the Effects of Glucocorticoids on Adhesion of PMNs to HUVECs. To determine whether the modulation of endothelial adhesiveness by dexamethasone was receptor-mediated we studied the effects of RU-486, a noncompetitive antagonist of glucocorticoid receptors (33, 34). RU-486 (10 μM) completely reversed the effect of dexamethasone on LPS-stimulated adhesiveness of HUVECs (Fig. 1, $P < 0.0005$). In other experiments 100-fold higher concentrations of the less potent steroid receptor agonist cortisol (IC_{50} = 100 nM, $n = 2$), but not its inactive metabolite tetrahydrocortisol (0.1 and 10 μM), diminished the LPS-stimulated increment in adhesiveness (data not shown). Further, neither indomethacin (10 μM) nor sodium salicylate (1 mM) affected the LPS-stimulated increment in endothelial adhesiveness.

Glucocorticoids Modulate Expression of ELAM-1 and ICAM-1 on LPS-Stimulated Endothelium. We next determined whether the effect of corticosteroids on endothelial cell adhesiveness resulted from diminished expression of ELAM-1 or ICAM-1. After stimulation by LPS, HUVECs increased expression of ELAM-1 by 321% ± 68% (SEM, $P < 0.0001$, n = 16) and ICAM-1 by 250% ± 38% ($P < 0.0001$, $n = 16$, Fig. 2).

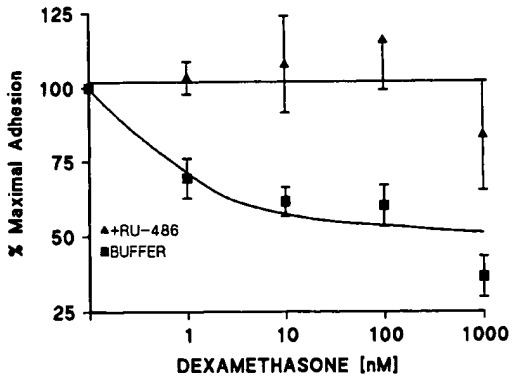

FIG. 1. Inhibition by dexamethasone (0.1–1000 nM) of PMN adhesion to LPS-stimulated HUVECs. HUVECs were stimulated, as above, in the presence or absence of various doses of dexamethasone and RU-486 (10 μM). Adherence of PMNs to unstimulated endothelium was determined and subtracted from the PMN adherence to stimulated endothelium and then expressed as a percentage of net adherence of PMNs to untreated endothelial monolayers. In the absence of dexamethasone RU-486 did not affect adhesion of PMNs to either unstimulated (122% ± 16% of control, $n = 4$) or LPS-treated endothelium (114% ± 11% of control, $n = 4$).

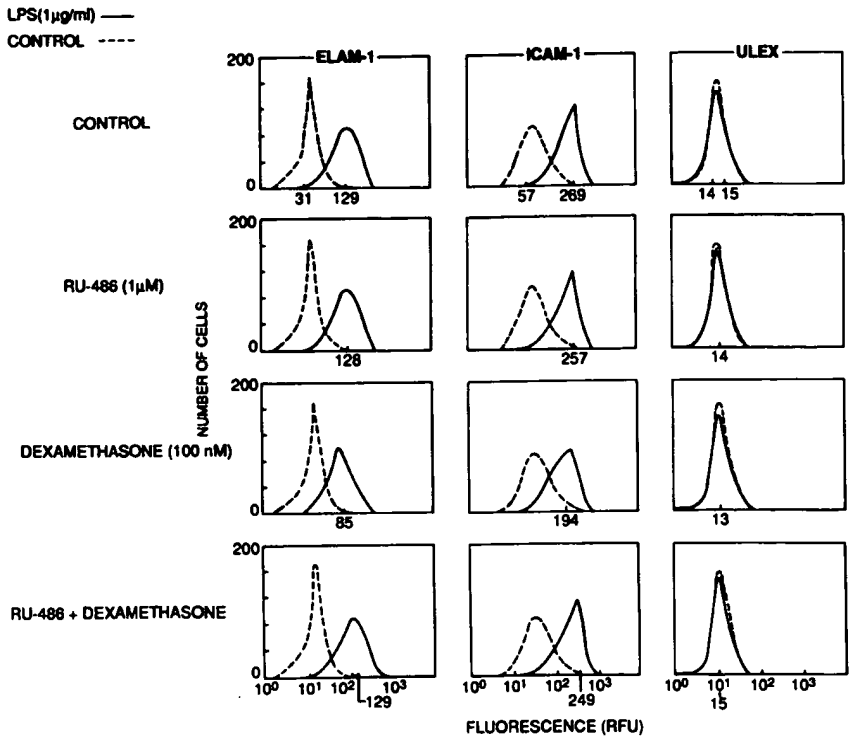

FIG. 2. Inhibition by dexamethasone of the increased surface expression of ICAM-1 and ELAM-1 by LPS-stimulated HUVECs. Fluorescence histograms are as follows: top row, resting and activated (LPS, 1 μg/ml) HUVECs stained with antibodies directed against ICAM-1, ELAM-1, and *Ulex europaeus* agglutinin I; second row, resting and activated HUVECs activated in the presence of RU-486 (10 μM); third row, HUVECs activated in the presence of dexamethasone (0.1 μM); and bottom row, resting and activated HUVECs activated in the presence of dexamethasone (0.1 μM) and RU-486 (10 μM). Mean fluorescence of cells stained with FITC-labeled antibody (MOPC-21) alone was 10 relative fluorescence units (RFU). Shown is a representative experiment of seven, the mean results of which are presented in Figs. 3 and 4.

Dexamethasone (IC_{50} < 1 nM) inhibited the LPS-stimulated expression of ELAM-1 and ICAM-1 ($P < 0.00001$ and $P < 0.00001$, respectively, Fig. 3) without altering basal expression of these molecules (data not shown). Dexamethasone did not alter binding of rhodamine-labeled *Ulex europaeus* agglutinin I to the endothelial cell surface (Fig. 2). Similarly, none of the compounds studied affected the nonspecific binding of antibodies MOPC21, UPC10 (isotype controls), or FITC anti-mouse IgG (data not shown). As expected, RU-486 completely reversed the effect of dexamethasone on the LPS-stimulated expression of ICAM-1 and ELAM-1 ($P < 0.004$, Figs. 2 and 4).

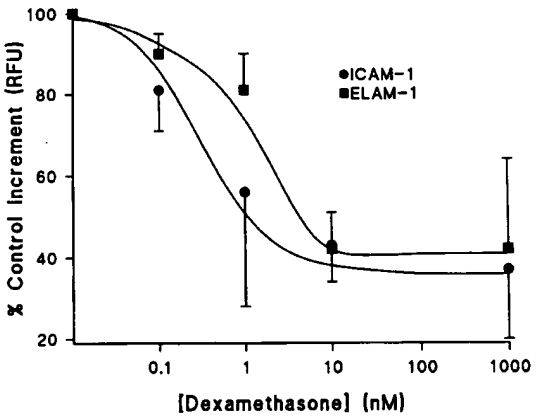

FIG. 3. Inhibition by dexamethasone (0.1 nM–10 μM) of the up-regulation of ICAM-1 and ELAM-1 expression on LPS-stimulated HUVECs. The results shown represent the means ± SEM of 4–10 experiments. RFU, relative fluorescence units.

Although RU-486 appeared to diminish LPS-stimulated up-regulation of ICAM-1 (Fig. 4), the difference observed was not statistically significant. Similarly, cortisol, but neither tetrahydrocortisol nor sodium salicylate, inhibited expression of ICAM-1 and ELAM-1 ($P < 0.003$, Fig. 5). None of the agents studied affected binding of *Ulex europaeus* agglutinin I to HUVECs (Fig. 2).

Glucocorticoids Prevent Accumulation of Message for ELAM-1 in Response to LPS and IL-1α But Not in Response to TNF. To further define the mechanism by which glucocorticoids inhibit up-regulation of adhesive molecules, we studied the effect of dexamethasone (0.1 μM) on the level of mRNA for ELAM-1 in HUVECs. Treatment of HUVECs with LPS, IL-1α (20 units/ml), and TNF (50 units/ml) stimulated a marked increase in detectable message for ELAM-1 in HUVECs (Fig. 5). Dexamethasone (0.1 μM) did not affect basal levels of mRNA for ELAM-1 but markedly inhibited the LPS- and IL-1α-stimulated increase in message. In contrast, dexamethasone did not affect the TNF-stimulated increment in detectable ELAM-1 message, an observation that suggests that glucocorticoids do not directly affect stability of message for ELAM-1. Although these findings need to be fortified by further studies of the effects of glucocorticoids on the stability of ELAM-1 message and the rate of transcription of ELAM-1 by stimulated endothelium, the results suggest that glucocorticoids act at the transcriptional level.

Dexamethasone Does Not Reverse the Effect of TNF-α on Endothelial Adhesiveness for PMNs. Since dexamethasone did not affect the level of message for ELAM-1 in TNF-α-stimulated HUVECs, we sought to determine whether this was reflected in the adhesiveness of TNF-α-treated endothelium for PMNs. As previously reported, TNF-α (50 units/ml)

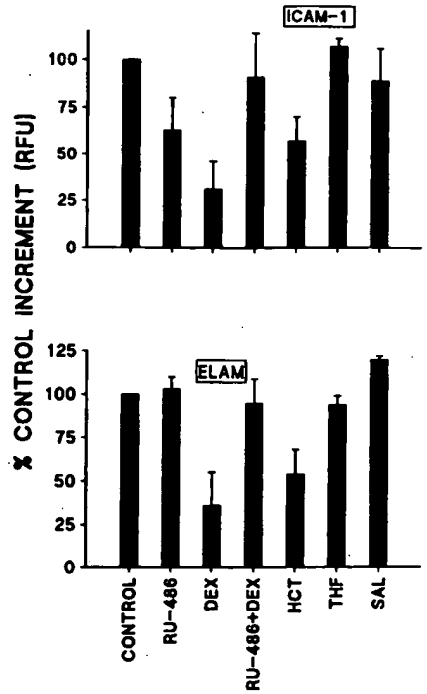

FIG. 4. Corticosteroids inhibit the LPS-stimulated expression of ICAM-1 and ELAM-1. HUVECs were incubated with LPS (1 μg/ml) in the presence of dexamethasone (DEX, 100 nM), RU-486 (10 μM), dexamethasone plus RU-486, cortisol (HCT, 10 μM), tetrahydrocortisol (THF, 10 μM), or sodium salicylate (SAL, 1.25 mM). (*Upper*) Effect of the various compounds tested on the LPS-induced increment in expression of ICAM-1 expressed as a percentage of the increment induced by LPS alone. (*Lower*) Effect of these same compounds on the LPS-stimulated increment in ELAM-1 expression. Shown are the means ± SEM of 3–10 experiments. RFU, relative fluorescence units.

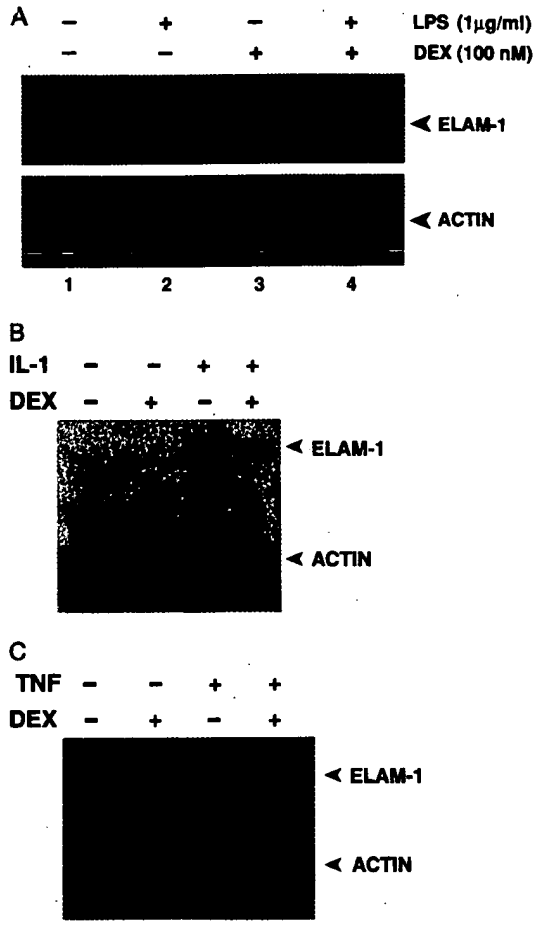

FIG. 5. Dexamethasone inhibits the stimulated increase in mRNA for ELAM. (*A*) HUVECs were incubated without (−) or with (+) LPS (1 μg/ml) in the presence (+) and absence (−) of dexamethasone (DEX, 0.1 μM). When normalized for the content of actin mRNA, LPS stimulated a 234% increase in mRNA for ELAM. Dexamethasone did not affect the basal level of mRNA for ELAM-1 (98% of control) but completely inhibited the LPS-stimulated increase in mRNA for ELAM-1 (90% inhibition). Similar results were found in a second experiment. (*B*) HUVECs were incubated with (+) or without (−) IL-1α (20 units/ml) in the presence (+) or absence (−) of dexamethasone (0.1 μM). When normalized to the content of actin mRNA, dexamethasone inhibited the IL-1α-induced increase in mRNA for ELAM-1 by 95% without affecting basal levels of mRNA for ELAM-1 (104% of control). Similar results were found in a second experiment. (*C*) HUVECs were incubated with (+) or without (−) TNF-α (50 units/ml) in the presence (+) or absence (−) of dexamethasone (0.1 μM). When normalized to the content of actin mRNA, dexamethasone did not affect either basal mRNA for ELAM-1 (97% of control) or the TNF-α-induced increment in mRNA for ELAM-1 (110% of control). Similar results were found in a second experiment.

rendered the endothelium more adhesive to PMNs (23% ± 1% adherence vs. 9% ± 2% adherence, $n = 4$, $P < 0.01$) and dexamethasone did not diminish the increased adhesiveness of TNF-stimulated endothelium for PMNs (21% ± 1% adherence, $n = 4$).

DISCUSSION

We show here that one important mechanism by which glucocorticoids may affect the inflammatory response is modulation of the capacity of the endothelium to respond to an inflammatory stimulus. Glucocorticoids, acting at their cytoplasmic receptors, diminish the LPS-stimulated increase in endothelial adhesiveness for resting PMNs and diminish transcription and expression of pro-inflammatory adhesive molecules on the surface of the endothelium. Since recent studies have increasingly pointed to the central role of the endothelium in directing the traffic of leukocytes into inflamed areas, our observations bear directly on the mechanism for the antiinflammatory effects of glucocorticoids. Moreover, our results help to explain the dramatic leukocytosis observed in patients taking therapeutic doses of corticosteroids [concentrations similar to those studied here (17)].

RU-486 stabilizes the association of steroid receptors with heat shock protein 90 in the presence of ligand, which prevents translocation of glucocorticoid receptors to the nucleus and thereby blocks transcription of genes containing glucocorticoid-responsive elements (33, 35–40). Our demonstration that RU-486 reverses the effects of dexamethasone on the adhesive qualities of HUVECs and the expression of adhesive molecules on their surface is therefore most consistent with the hypothesis that the effects of steroids on the endothelium are mediated through glucocorticoid receptors. Further confirmation is provided by the observation that cortisol, but not its inactive metabolite tetrahydrocortisol, inhibits endothelial cell responses to LPS. The absence of a glucocorticoid-responsive element in the gene for ELAM-1 suggests that glucocorticoids must either interfere directly with a transcriptional regulator of ELAM-1 transcription or induce the synthesis of a second regulatory element. Montgomery and co-workers (41) have demonstrated that NFκB regulatory elements are necessary (but not sufficient) for transcription of ELAM-1. Thus the induction by human glucocorticoid receptor (hGR) of the synthesis of a counterregulatory element such as IκB could

account for the inhibition of ELAM-1 expression by dexamethasone. Steroid–receptor complexes also participate in protein–protein interactions with *jun*, preventing its interaction at AP-1 regulatory sites of the 5′ flanking regions of appropriate genes (36, 37), although the AP-1 site present in the gene for ELAM-1 does not appear to participate in the regulation of ELAM-1 (41).

Our results appear to differ from those of Bochner *et al.* (5), who found that prolonged (24 hr) treatment of microvascular endothelium from foreskin with glucocorticoids did not prevent LPS from modulating PMN adhesion, whereas, as we report here, treatment of HUVECs for 4 hr dramatically diminished adhesiveness. The inefficacy of prolonged treatment with corticosteroids may have been due to "desensitization" of hGR (i.e., complete depletion of hGR in the cytosol) in the presence of high concentrations of agonist (42). Alternatively, occupancy of hGR may only transiently transactivate genes that regulate ELAM-1 transcription or translation, permitting subsequent activation of ELAM-1 transcription by LPS.

We were surprised to observe that dexamethasone did not inhibit accumulation of mRNA for ELAM-1 induced by TNF-α. In parallel studies Ghezzi and Sipe (43) found that dexamethasone inhibited LPS-stimulated, but not TNF- or IL-1-stimulated, serum amyloid protein A secretion in mice. Thus LPS, TNF, and IL-1 may induce the transcription of ELAM-1 by several different mechanisms, only some of which are sensitive to corticosteroids.

These experiments permit us to suggest a mechanism for the antiinflammatory effects of glucocorticoids: acting via their receptor, glucocorticoids prevent the recruitment of leukocytes at inflammatory loci by inhibiting the display of adhesive molecules on the surface of the endothelium. This hypothesis rests on three separate lines of evidence: *functional*, at nanomolar concentrations glucocorticoids inhibit endotoxin-stimulated increases of endothelial adhesiveness for leukocytes; *phenotypic*, at similar concentrations they inhibit the stimulated expression of adhesive molecules on the surface of HUVECs; *genotypic*, glucocorticoids inhibit accumulation of mRNA for ELAM-1 in endotoxin- and IL-1-stimulated cells. In addition to these direct receptor-mediated effects of glucocorticoids on the capacity of HUVECs to localize the inflammatory response, glucocorticoids also inhibit the ligand-induced release of cytokines (IL-1, IL-3, and TNF-α) by endothelial and other inflammatory cells (5–12). These findings suggest the general hypothesis that corticosteroids act as antiinflammatory agents by diminishing, directly and indirectly, the ability of HUVECs to direct leukocyte traffic into inflamed or infected tissue. Our data also provide a reasonable explanation for the opposing effects of endotoxin and glucocorticoids in infection and immunity (15, 16).

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